The Journal of Parasitology

Volume 6 DECEMBER, 1919 Number 2

NOTES ON NORTH AMERICAN MYXOSPORIDIA*

HENRY B. WARD

In this paper are published data on three new species of Myxosporidia. Two species were observed in Lake Erie as parasites of a minnow; the third came from a species of Pacific salmon. Both cases present some unusual features that seem worthy of record. I am greatly indebted to my colleague, Dr. R. Kudo, for valuable assistance given while I was working up the material. The beautiful sketch (Plate V) illustrating the species from Lake Erie was prepared by Mrs. H. S. Jennings, to whom my thanks are due for the courtesy.

Myxobolus aureatus nov. spec. (Plate V)

Host: *Notropis anogenus.*
Location: between the fin membranes.
Locality: near Put-in-Bay, Lake Erie.

Some years ago while engaged in the study of fish parasites for the U. S. Bureau of Fisheries, I discovered a case of infection with a sporozoan parasite which, on examination, proved so unusual in character that careful studies were made of the material then available. The notes made at that time were laid aside in order to secure further specimens and to work out the entire life history. It has proved impracticable as yet to repeat the study of fresh material on the spot and the importance of the find leads me to prepare the data for publication in order that the attention of others may be directed to the species. The form studied departs in some respects from all Myxosporidia yet described and commands attention for certain peculiar biological features.

In August, 1898, while I was seining near the hatchery of the U. S. Bureau of Fisheries at Put-in-Bay, Ohio, some minnows were taken which attracted immediate attention by virtue of their striking appearance. Several species of Notropis were netted in the same locality and all were carefully examined. One was conspicuous and only that one was infected in any way. The species in question was

* Contributions from the Zoological Laboratory of the University of Illinois, No. 145.

determined by Dr. W. C. Kendall, who was serving as ichthyologist of the party, as *Notropis anogenus,* although he noted that these individuals were young and did not agree in all details with the description of that species. These minnows were 2 to 3 centimeters long measured without the caudal fin. In all, thirty specimens of this minnow were captured and seven of these were infected with a myxosporidian parasite. The infected specimens were not inferior in size or vigor to the others of the same species. Even those most severely attacked by the parasite manifested normal activity and responded to experimental stimuli as promptly and accurately as those which showed no sign of being infected. The specimen which was most heavily infected was the most vigorous of all the minnows taken. It lived more than twenty-four hours in a small dish only 4 inches in diameter without any change of water and when killed was still very active.

The infection was markedly conspicuous. At first glance one could see one to many small cysts in the membrane of the fins. They lay between the ectodermal layers of the fin membrane, appearing as brilliant opaque points in the otherwise delicately transparent organ. The cysts were particularly conspicuous because of their striking coloring. Each appeared as an oval body perfectly opaque and glittering like a mass of metallic gold. These cysts were absolutely confined to the fins. Nowhere else on the surface of the body could there be seen even a single such structure and careful dissection failed to disclose any in the flesh elsewhere. Nor were any structures found which could be associated with them even as modified cysts or as developmental stages of the organism. This single stage in the location designated was the only phase in the life history of the organism that I was able to discover. Of the unique character of the location and the color, I shall say more later.

The number of such cysts in the individual case varied widely. In one specimen only a single cyst was present. That was located in the anal fin. In most specimens the cysts were fairly numerous. The individual represented in the plate (Fig. 1) shows the average frequency of infection. It had about thirty-five cysts, distributed as follows: two cysts in the dorsal fin, four in the caudal, eight in the anal, four and two in the two pectoral fins, and three in the single ventral fin present, one of the ventrals being missing in this specimen. The most heavily infected individual had about forty cysts; six of these were located in the dorsal fin, five in the anal, ten in the left pectoral and six in the right pectoral, five in the left ventral and seven in the right ventral. The various specimens showed most distinctly that no uniformity of distribution obtains either with regard to the degree of infection in any particular fin or in respect to the fins infected. Careful

examination of the specimens showed that both the paired and the unpaired fins were infected; the right and left sides proved to be variably infected and any one of the fins might be free from infection although the others were at the same time heavily infected.

In most cases the cysts were clearly separated from each other, though in a few instances they were apparently connected. Even here careful examination of the region under appropriate magnification demonstrated the fact that cysts overlapped in profile only and were actually separate from each other. The cysts were usually single and well separated from those nearest, but in some cases a fin carried a group of two to six cysts rather closely grouped together. There seemed to be no regularity in the occurrence of these groups and as already indicated they were in reality separate cysts though appearing on superficial examination to form a connected mass. As they increased in size the cysts seemed to accumulate chromatophores on the surface. At an early stage when the cyst was small, the chromatophores were few in number; later as the cyst increased in size the chromatophores became much more numerous, and in the largest they were thickly strewn over the surface. Such differences were often seen in adjacent cysts in the same group where the mass of chromatophores imparted a darker, heavier aspect to the older cyst.

The examination of these cysts under a higher magnification showed some interesting structural features. They were exceedingly regular in form and fairly uniform in size although the latter appeared to vary a little with age and development. The individual cyst was a smooth margined ellipsoid, measuring from 1 to 1.6 millimeters in larger diameter and from 0.8 to 1.2 millimeters along its transverse axis (PlateV). The striking color of the living cysts has already been mentioned. Under a high power it seemed to be a clear orange yellow, but under all circumstances was perfectly opaque. The surface of the cyst was spotted with conspicuous black patches of minute size. These spots lay on the outer surface of the cyst wall and were in reality the chromatophores of the skin, but they were distinctly more abundant here than elsewhere in the fin or on the body skin of the minnow. The gilt color was contained in the cyst wall itself, as was easily demonstrated on pulling the structure to pieces. This color faded slowly in alcohol and formol, first losing its brilliancy and later disappearing entirely, leaving the cyst wall a dull white or grayish tone. The cyst wall was noticeably tough and thick in spite of the insignificant size of the cyst. When the wall of the living cyst was torn apart by needles, there exuded a milky white mass from the interior consisting chiefly of the spores to be described later. The gilt color and opacity of the wall remained unchanged.

The presence of color is very unusual in myxosporidian cysts. So far as I can ascertain it is shared by no other species yet described. Recently Southwell and Prashad (1918) reported a cyst of *Myxobolus nodularis* in the muscles of *Rasbora daniconius* as of a creamy yellow color, "in one case appearing blackish owing to the large number of black granules scattered in its surface." These granules are very probably chromatophores on the surface and belong to the host tissue as is indicated in descriptions of other species by various authors; if this inference be correct their record is in part similar to that described here. But the color can hardly be comparable. In fact, as the authors just quoted describe cysts of another species (*Myxobolus rohitae*) in the gills of *Labeo rohita* as "of a creamy yellow color," it seems as if they were describing a shade or tone in the preserved specimen rather than a distinct color or pigment; moreover, there is nothing to indicate that the color belongs to the cyst and not to the contents or to the host tissue.

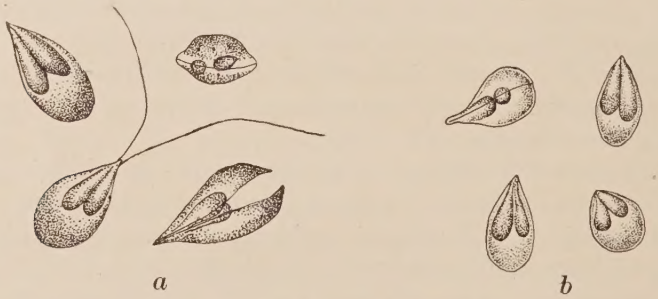


Fig. A.—Spores of *Myxobolus aureatus* drawn from fresh material. *a*, × 1300; *b*, × 972.

In discussing such structures various authors agree in stating that the color of the cyst belongs to the host tissues and can be found on examination to disappear when the cyst is removed, thus showing that the true cyst wall is colorless. That is certainly not the condition which obtains in this case, for the color belongs to the cyst and cannot be separated from it in life. In section, the protoplasm shows a poor differentiation into ectoplasm and endoplasm. The former, granular and recticular, covers the entire surface as a thin layer, while the latter is highly vacuolated, containing only mature spores.

When the cyst wall is torn open there exudes a milky white mass composed primarily of the spores. These are characteristic in appearance and demonstrate immediately the myxosporidian nature of the cyst. The spores are ovoid in form (Fig. A), slightly pointed at one end and rounded at the other. The pointed end is the capsular pole. There is no caudal filament present. From one aspect the spore appears

slightly narrower and more pointed than when seen at right angles. Up and down the narrow aspect the spore shows a distinct ridge which marks the line of separation between the two valves of which the spore wall is composed. When the material is left standing in water, the valves separate along this line (Fig. *A, a*) and are seen to be perfectly symmetrical and similar in all respects. The shell is of moderate thickness and bears a flange at the lower non-capsular pole. The greatest convexity of the valve is located two-thirds of the way from the pointed pole. The spores vary in length from 12.4 to 13.5μ with a breadth of 6.5 to 7.5μ, and an average thickness of 5μ.

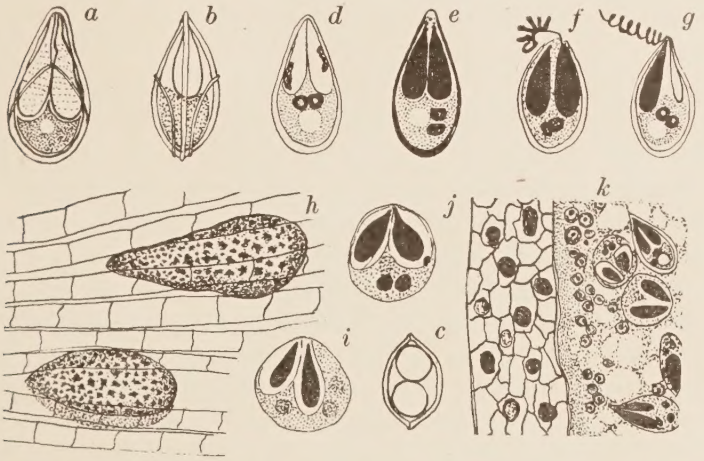


Fig. B.—*Myxobolus aureatus;* magnified 1,500, except as otherwise stated. *a, b, c,* unstained preserved spores in different views; *d, e,* stained mature spores; *f, g,* spores with extruded coiled polar filament from section; preparation stained with Giemsa; *h,* a portion of the caudal fin showing two cysts, × 22; *i, j,* young spores, stained; *k,* a portion of the cross-section of the fin, showing the peripheral part of the parasite, × 900.

Each spore contains two capsules, located in the pointed half of the shell. They are not always exactly alike, for frequently one is slightly longer than the other or else located a little further from the actual pole so that its inner end lies in a different plane from the other. These capsules are elongated pyriform in outline and measure 6 to 7 or rarely 7.5μ in greatest length. Ordinarily the filament is not extended, but it can be made to appear by letting the spore stand twenty-four hours or more in plain water. Then one sees two extremely delicate threads, one from each capsule, extending into the water a distance of one and one-half to two times the major diameter of the spore (Fig. *A, a*). In the preserved material they may be forced out (Fig. *B, f, g*) in such fashion as to indicate six or seven coils in the filament. The binucleated

finely granular sporoplasm shows always an iodinophilous vacuole which becomes distinctly contoured by taking a deep brownish color when the spore is treated with Lugol's solution. Its diameter is about 2μ.

The characteristics of this species as described above cause it to fall clearly within the family of the Myxobolidae of Thélohan and the absence of any caudal filament on the spore membrane places it in the genus Myxobolus of Bütschli. The polar capsules are equal in size as in the type species *Myxobolus mülleri* Bütschli of Europe which infests many fresh water fishes. Its inclusion in this genus emphasizes its relationship to *M. pfeifferi,* the cause of the devastating barbel disease, and to *M. cyprini,* which gives rise to the destructive fish-pox of the carp.

Only a few forms of the Myxosporidia have been reported within the limits of the United States. Gurley (1893; 111) listed nine species of which eight occur in fresh water hosts. A little earlier Linton (1891) had described a specially interesting form from fresh waters. The species infected was *Notropis megalops* and the locality from which they came was the Black River, Lorain County, Ohio. Since the host is a minnow closely related to that on which occurred the species described in this paper and since the localities are only a short distance apart on the south shore of Lake Erie, one is tempted to ask if the two parasites are not identical. A close examination of Linton's record and figures shows that they cannot possibly be the same species. Linton describes his form as producing globular or botryoidal masses on the side of the head and body and at the base of the fins. The illustration demonstrates clearly the distribution in groups or clusters and further the location of these masses on the body wall at the base of the fins. They occur in the specimen he figured at the base of the pectoral, ventral, anal, dorsal, and caudal fins, but in no case do they encroach on the membrane of the fin itself. They are confined exclusively to the surface of the body proper. The masses are made up of cysts that are distinctly confluent and in no case figured was one cyst discrete and separate from other cysts. The component cysts vary from two to three millimeters in diameter. Finally Linton describes the color of these cysts as white with minute patches of black pigment belonging to the skin of the host.

When these data are compared with those already given for the Put-in-Bay species the differences are conspicuous. The cysts of the latter species are usually single and even when grouped one can distinguish them as separate and entirely unconnected masses. They never form clusters or groups of a botryoidal character. In size they are only

one-third to one-half the dimensions of those in Linton's specimens. In general the cysts described by Linton are much more nearly spherical than those in the present species. Since Linton's material was not living when submitted to him it is uncertain what the appearance was in life, but in the letter from Mr. McCormick of Oberlin College that accompanied the specimens and described their capture no note is made of any color in the living material. It is certainly difficult to believe that any collector could overlook the very brilliant and striking color of the cysts of the Put-in-Bay species so that one may reasonably infer the absence of such coloring. The hosts are different species, one being a river form and the other a lake species, and the lake form was also much smaller than the other for which Linton records a length, exclusive of caudal fin, of 47 to 57 millimeters.

But the most striking difference is found in the location of the cysts. In the Put-in-Bay species they are always in the membranous expansion of the fins and never on the surface of the body, whereas, in Linton's species as already described the location is precisely the contrary. This difference in distribution is uniform and unvarying. No single exception is recorded for either species. The location of the cysts in Linton's species is not uncommon, although most forms that occur on the surface of the body are not confined so rigidly, as his figure indicates this form to be, to the region of the skin just at the base of the various paired and unpaired fins. But the new species described here is found only within the fin membrane, a most unusual location. The significance of this is discussed later, but the marked and constant difference in the location of the cysts may be regarded as clear evidence of the specific difference of the two parasites.

When the spores of the two forms are compared, one finds similar differences. They are, to be sure, much alike in general appearance and structure, but these features are merely those characteristic of all spores in this genus of Myxosporidia. If the drawings of Linton's spores are all of approximately the same magnification as is indicated in the explanation of his plate, then those spores vary in size far more than these. He states the dimensions of the spores in that species as 17μ long, 10μ broad, and 6μ thick, which makes them distinctly larger and different in proportions. They are more drawn out and show a concave taper wanting in the spore from the Put-in-Bay minnow. No comparison can be made of internal structure as he was unable to make out the polar capsules, threads, or nuclei in the spores. In view of all these features it is impossible to include the Put-in-Bay form in the species described by Linton.

The species of Myxobolus parasitize the gills, fins, scales, spleen, kidney, and muscles of the host. Commonly they are found in the

connective tissue of these organs and occur in several parts of the body. In our case the very specific localization of the parasite is distinctly noteworthy and in this the species differs from all others in the genus so far as is shown in literature available. The occurrence of Myxosporidian cysts in the fins of fishes is rare indeed. Minchin (1903: 339) cites only five cases: *Henneguya linearis* (Gurley) in *Ameiurus melas* at the base of the dorsal fin; *Myxobolus oviformis* Thél. in the fins, gills, kidney, and spleen of *Gobia gobio; Myxobolus mülleri* Bütschli from the fins and gills of *Leuciscus cephalus; Glugea acuta* Thél. from the connective tissue of the dorsal fin in *Nerophis aequoreus,* and from the same region in *Syngnathus acus.* From the same genus as our host species Minchin records only one case of infection and that in the skin of *Notropis megalops,* the case described by Linton (1891) and discussed elsewhere in this article. Careful examination of the literature shows that seven cases described as fin infection have been reported up to the present of which, except *Myxobolus seni,* all infect also other organs of the host. These species are as follows:

Myxobolus sp. Müller (1841: 480)
Myxobolus oviformis. Thélohan (1895: 351)
Myxobolus volgensis. Reuss (1906: 200-201)
Myxobolus gigas. Parisi (1912: 293-294)
Myxobolus seni. Southwell and Prashad (1918: 347)
Henneguya linearis var. Gurley (1893: 417)
Henneguya nüsslini. Schuberg and Schröder (1905: 56)

Of the four cases from the same region in the host, cited after Minchin, the last two concern marine fishes, and the first is doubtful. Of this case, Gurley (1893: 417) speaks as follows in the original description of the species: "In cysts *at the base* of the dorsal fin of *Ameiurus melas* Raf. from Storm Lake, Iowa, a spore occurs which I strongly suspect to be identical with this species, as it answers in every respect to the rather meager diagnosis." As the cyst is *below* and not *in* the fin, the location of the parasite does not at all correspond to that of our species. Gurley also in speaking of *M. linearis* (1893: 416) writes, "Cysts invariably* embedded in the subcutaneous tissue of some part of the head (especially the under surface of the lower jaw) of *Hybognathus nuchalis* Ag." Here again the location is not that of the species under consideration, as in the former case the cyst is really in the body near the base of the fin. Only one reference in the literature seems to agree in part with the description of *M. aureatus.* Southwell and Prashad (1918: 347) mention that cysts of *Myxobolus seni* were found only "on the median and caudal fins of *Labeo rohita,*" a species of fresh water fish taken in Mirpur, India. This is the only species of Myxobolus really and exclusively located *in* the fin. Unfortunately

*Among several hundred cysts one was seen *at the base* of the pectoral fin.

the authors have given only a very scanty description of this parasite. It is certainly different from the species described here and a detailed comparison is unnecessary.

Henneguya brachyura nov. spec.

Host: *Notropis anogenus.*
Location: in the cartilaginous fin ray.
Locality: near Put-in-Bay, Lake Erie.

In studying sections of the caudal fin of one of the minnows that was infected by *Myxobolus aureatus,* a species of *Henneguya* was found encysted in the fin ray. The cysts were rounded with slightly irregular contour and imbedded in the ray. In size they varied from 160μ in diameter up to 360 by 240μ. No particular cyst membrane could be recognized. The differentiation of the protoplasm into ectoplasm and endoplasm is distinct. The ectoplasm constitutes a layer 4 to 6μ thick, covering the entire surface of the parasite; it shows a

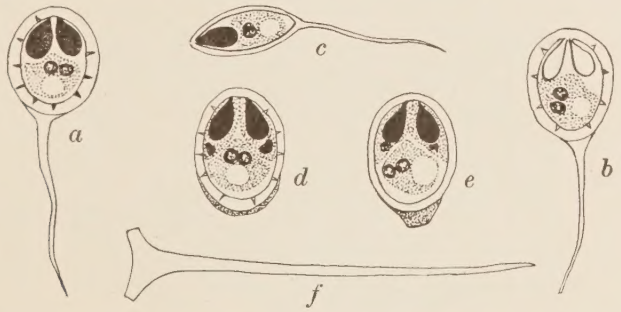


Fig. C.—*Henneguya brachyura* × 1,500, except *f*. *a, b, c,* different views of stained spores from section; *d, e,* young spores developing the tail; *f,* detached tail in a section, × 3,560.

very finely granular structure. The endoplasm is coarsely alveolar and filled with mature spores in the central portion, while numerous nuclei and young spores in various developmental stages are present in the peripheral portion.

The spore (Fig. *C*) is a rounded oval in front view but spindle-shaped with symmetrically built valves in profile. The shell is rather thick and the sutural ridge fairly well marked, the sutural edge exhibiting a variable number of folds (8 to 10). The pyriform polar capsules are usually of the same size and form. The tail is a single process, usually more or less bent or irregularly curved, very rarely being straight. In general it is sinuous with two or three shallow curves (Fig. *C, a*) and is rather short, tapering gradually to a point. In young spores which are less deeply stained by any stain, various developmental stages of the tail are easily recognized (Fig. *C, c, d*).

Giemsa solution stains the shell proper a clear blue, while the tail takes on a beautiful pink color, showing a distinct difference in affinity for dyes between the material in the tail and in the shell. According to Gurley (1894: 250), the tail of *Henneguya macrura,* with which the present species is closely related, was completely dissolved by concentrated sulphuric acid. It seems probable that the tail of this type is entirely different in its development from that of the ordinary bifurcated type, but further studies could not be made in this species owing to the small number of parasites available. In section, dimensions of the species are: length, 10 to 11.5μ; breadth, 8 to 8.75μ; thickness, 4 to 5μ; polar capsules, 3 to 4 by 2μ; length of the tail up to 17μ.

Among the known species of *Henneguya, H. macrura* Labbé (Gurley, 1894: 250) seems to be most closely related to the form under discussion. A comparison of two forms yields the following data:

	H. macrura	The Present Form
Habitat	Subcutaneous connective tissue Head of *Hybognathus nuchalis* Neches River, Texas, November, 1891	Fin ray of cadual fin; *Notropis anogenus;* Put-inBay, Ohio, August, 1898
Cyst	Large, elongated; size up to 6 by 2 mm.	Very small; invisible to naked eye; size in section, up to 360 by 240μ
Spore, similar features	Rounded oval; length 10 to 11μ, breadth 6 to 8μ, thickness 4μ, length of tail 30 to 40μ	Rounded oval; length 10 to 11.5, breadth 8 to 8.75μ; thickness 4 to 5μ; polar capsules 3 to 4 by 2μ; length of tail up to 17μ
Differences in the two spores	Sutural ridge without any folds; tail longer, slightly bent; polar capsules larger; valves very unequal	Sutural edge with distinct folds; tail shorter; sinuous; polar capsules smaller; valves usually equal

From this comparison it appears that in form and size the two spores are in close agreement, but the polar capsules differ very distinctly and the valves of the spore are rather sharply contrasted by their nearly equal form in the present type and their unequal form in the older species. Furthermore, the tail of the new form is only half as long as that in *H. macrura* and shows a wavy outline with two or three shallow curves instead of a simple, flat curve as in *H. macrura.* When one adds to these features which distinguish the two spores the radical difference in the size of the cysts, too great to be explained on the basis of differences in age and growth, it is hard to include both in the same species.

Finally Gurley emphasizes the location of the cysts, saying that in *H. macrura* the cysts are "almost invariably situated on some portion of the head," and stating that he had seen "but one exception, a cyst situated at the base of the pectoral fin," whereas the species under consideration was found actually at the opposite end of the body and

only in a cartilaginous ray of the caudal fin. I should not neglect to mention also the difference in hosts and the occurrence of the two parasites in separate geographic provinces. In connection with the morphologic evidence these facts are of significance in contrasting the two forms.

For these reasons I have decided that the new form cannot be brought under the older designation and propose for it the name *Henneguya brachyura*.

Henneguya salminicola nov. spec.

Host: *Oncorhynchus kisutch*, the silver salmon.
Location: connective tissue in body muscles.
Locality: taken in Stickeen River, S. E. Alaska.

In connection with studies I am carrying on with the Pacific salmon, the U. S. Bureau of Fisheries sent me preserved specimens labeled

Fig. D.—Cysts of *Henneguya salminicola* in body muscles of Pacific salmon. Approximately half natural size. Preserved specimen.

"Pieces of Salmon with Cysts," collected by E. Lester Jones, Stickeen River, Alaska, about Sept. 10, 1914. Dr. Jones, who was at that time Deputy Commissioner and engaged in a trip to inspect conditions in Alaskan waters, received the fish within twelve hours of the time they were taken in gill nets so that they were in good condition. The saline solution in which they had been preserved was of a density of 5 or 6 per cent. and had kept the specimens passably well.

On examining the specimen (Fig. *D*) the observer was at once struck by the pale, whitish flesh around the cysts in clear contrast with the bright pink muscle usual in this fish. The zone of faded tissue surrounded the cysts to a width of 6 to 8 mm. The cysts themselves were pyriform, fairly uniform in size, and hard to the touch. They measured from 3 to 6 mm. in diameter. These cysts were especially conspicuous because some were pendant from the peritoneal wall, and

projected into the body cavity. They are not generally superficial in location as cysts appear everywhere through the muscle mass from the subperitoneal to the subdermal connective tissue, though of course all are subperitoneal in position. While they occur in groups in a certain sense, each cyst is entirely independent of those near it so far as can be determined by the unaided eye or by dissection. They certainly do not form botryoidal masses such as are found in some cases.

Sections demonstrate that the cysts are surrounded by a heavy capsule of connective tissue. Spores in various stages of development occur within the capsule and the mature spores are thickly massed together in the central area of the cyst.

Fig. E.—Spores of *Henneguya salminicola* from section of cyst stained with iron hematoxylin. × 1,180.

There was, of course, no chance to study living material. The form of the spores is clearly shown in a drawing (Fig. *E*) made from a section of the cyst contents; the slide had been stained in iron hematoxylin and picric acid. The two long and delicate spines that project from the non-capsular end of the spore are in reality prolongations of the shell that are not pierced by the cavity of the spore. This form is characteristic of the genus Henneguya. Here the caudal spines are separate throughout their entire length but are roughly parallel and not divergent. The two nearly equal polar capsules are not contiguous along the median line but are separated by a band one-third to one-half the width of a capsule. A large iodinophilous vacuole, 3.4 to 4μ in

diameter, is conspicuous in the spore, but the polar filament coiled in the capsules cannot be distinctly seen in the preserved material.

A series of careful measurements was made of the spores and their processes. The body of the spore when measured in stained specimens "over all" varied in length from 11.97 to 14.25μ, on the average being 12.42μ, though the norm of length as calculated from the series was very close to 12μ. If measured to the base inside, stained specimens are 8.4 to 8.66μ. The width of the body of the spore varied from 7.12 to 8.43μ, with an average of 7.92μ and a norm of 8μ. The length of the tail was from 30.78 to 38.19μ, with an average of 34.54μ and a

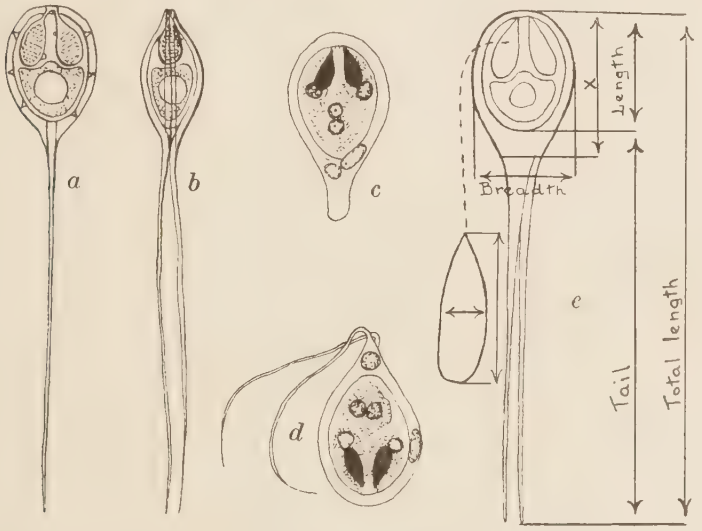


Fig. F.—*Henneguya salminicola; a, b,* unstained preserved spores; *c,* young spore; *d,* more advanced stage from smear stained with Giemsa; *a-d,* $\times$ 1,500; *e,* diagram showing limits observed in taking measurements, *x,* the length "over all," is not often used since the lower limit is not definitely marked.

norm of 35μ. In another set of thirteen specimens the average length was 12.44μ, the length of the tail 35.49μ; the average width of seven specimens was 7.63μ and the thickness of six specimens averaged 4.78μ.

The polar capsules range from 3.70 to 4.56μ in length by 1.59 to 2.85μ in breadth, or in the norm 4 by 1.8μ. One is almost always a little larger than the other, the difference being constantly about 0.25μ in length and half as much in width. Very few exceptions to this rule were met in a long series of measurements.

Since differences actually are found between measurements of spores of the same species in stained and unstained preparations, I give a table showing results obtained by two observers with different technic. The diagram (Fig. *F, g*) shows the limits used in making the measure-

ments recorded. A series of twenty-four to thirty mature spores was used in each case.

MEASUREMENTS OF SPORES OF HENNEGUYA SALMINICOLA

		Ocular Micrometer	
Measured by	Camera drawings	Giemsa	Unstained
Stained by	Iron hematoxylin		
Total length	42.75-52.44	43.5 -53.0	51 - 57
Length of spore body "Over all"	11.97-14.75	.....	.....
Length of spore body Inside base	.....	8.4 - 8.66	8.6 - 9.5
Length of tail	30.78-38.19	34.25-36.75	.....
Breadth of spore body	7.12- 8.43	7.5 - 8.5	8.6 - 9.5
Polar capsule Length	3.7 - 4.56	3.5 - 3.8	3.5 - 4.0
Polar capsule Breadth	1.59- 2.85	1.7 - 2.2	2.0 - 2.5

All measurements expressed in microns.

In form and size of spores this species resembles most closely *Henneguya zschokkei, H. schizura,* and *H. nüsslini. Henneguya nüsslini* was discovered in the trout by Schuberg and Schröder (1905), from whose description the following data are excerpted. The two cysts found lay in the subcutaneous connective tissue at the base of the dorsal fin. The spores were 12μ long by 8 to 9μ broad, and with the tail measured 32μ over all. The tail was split, but the two spines were never separated throughout their entire length. The polar capsules were 5μ long and 3μ wide; they do not meet along the median line, but are separated by a distinct space. The spore is rounded at the anterior end. In this respect and in the separation of the polar capsules the new species is like *H. nüsslini* and unlike the other species named above, but a comparison of the dimensions quoted shows that *H. nüsslini* has larger polar capsules and a larger spore body, whereas the total length is much less than in *H. salminicola.* These differences are too great to permit including the new form in the species *H. nüsslini.*

Henneguya schizura was first described by Johannes Müller but was not named until Gurley (1893:417) called it *Myxobolus schizurus.* The parasite is found only in the orbit, encysted in the connective tissue of the eye muscles, in the sclerotic and between the latter and the choroid. It occurs in young *Esox lucius* and is present in May and June. Müller looked for it without success in specimens of the pike from North America. The two species agree in the length of the spore body (12μ), but in *H. schizura* the spore is only 6μ broad as against 8μ here, and the tail in the former is three to four times as long as the spore body, whereas here it is barely three times as long. The size of the spores is sufficient in fact to distinguish this form from the new species described here although the peculiar and restricted distribution of *H. schizura,* occurring only in the orbital tissue, precludes the possibility of considering the new species identical with it.

A close resemblance exists between the new species and *Henneguya zschokkei* which was first described by Zschokke and named by Gurley

(1893). It inhabits the subcutaneous and superficial connective tissue in the trunk muscles of *Coregonus fera*. The cysts are round or oval and of considerable size, up to 30 mm. in maximum. The spore body is 10μ long by 7μ broad. The tail is four to five times as long as the spore body and is composed of two slightly curved and diverging spines. In comparison with the species from the Pacific salmon, *H. zschokkei* has a smaller spore body and a longer tail. In the former species the tail filaments are nearly parallel and never divergent as in *H. zschokkei*. These points form an adequate basis for separating the two forms.

It is worthy of note that such parasites though common in many types of fish are almost entirely unknown in salmon of any sort. An examination of the literature shows only a single record of a Myxosporidian parasite in any European salmon. That is *Lentospora cerebralis* which is the cause of the gid disease (Drehkrankheit) of young salmon in the first year of life.

I have not been able to find a single record of the occurrence of Myxosporidia in an adult Europan salmon and not one in a salmon of any age from this continent. During the last fifteen years I have examined personally several thousand Pacific salmon of all species and have never seen one infected so far as could be detected with the unaided eye. In searching for diseased fish I have been aided by a large number of fishermen and other cannery employes who knew I was anxious to secure all such specimens and were desirous of aiding me so that the cases I have recorded represent those culled from several hundred thousand fish, and there is no entry in my notes of a Pacific salmon affected with any sort of myxosporidian disease.

It is hardy possible that this case could represent a seasonal disease which fell outside the time limits of my experience, for I have collected salmon in the Alaskan coastal waters at least as late as September 1, and the date of this find was only ten days later. Further, no report of such a condition has been transmitted to me by the many men in that region who have been interested in my work and anxious to participate in it.

Finally, one must consider the chance that this is a localized disease and infects only or chiefly the salmon that run in the Stickeen River. I have not collected or studied the Pacific salmon in that precise region and so cannot venture to pass judgment on the question. But if the infection is localized it must be held within narrower limits than are usually observed by the parasites of migratory or marine fish so far as I know them; for I have studied the salmon run both north and south of the Stickeen River and the channels connecting with it, and the fish boats which supplied the canneries at which I was working ranged nearly as far as the Stickeen; yet no fish were seen with a similar infection.

So far as I can ascertain, this is the first published record of the occurrence of a myxosporidian parasite in any fish from Alaskan waters. While the lack of records is very likely due in part to the limited attention paid to diseases of fish from that region, I am also inclined to believe, from my own observation, that myxosporidian parasites are rare in fish found in Alaskan coastal waters.

Papers Cited

Gurley, R. R. 1893.—On the Classification of Myxosporidia, a group of protozoan parasites infecting fishes. Bull. U. S. Fish Comm., 11: 407-420.

1894.—The Myxosporidia, or Psorosperms of Fishes, and the epidemic produced by them. Rep. U. S. Fish Comm., 26: 65-304.

Keysselitz, G. 1908.—Die Entwicklung von *Myxobolus pfeifferi*. Arch. Protist., 11: 252-308.

Linton, E. 1891.—Notice of the Occurrence of Protozoan Parasites (Psorosperms) on Cyprinoid Fishes in Ohio. Bull. U. S. Fish Comm., 9: 359-361.

Mercier, L. 1909.—Contribution à l'étude de la sexualité chez les Myxosporidies et chez les Microsporides. Mém. class. sc. Acad. roy. Belg., 2: 3-30.

Parisi, B. 1912.—Primo contributo alla distribusione geografica dei missosporidi in Italia. Atti soc. ital. sc. nat., 50: 283-290.

Reuss, H. 1906.—Neue Myxosporidien von Süsswasserfischen. Bull. Acad. Imp. Sc. St. Petersburg, 25: 199-205.

Schuberg, A., and O. Schröder. 1906. Myxosporidien aus dem Nervensystem und der Haut der Bachforelle (*Myxobolus neurobius* n. sp. and *Henneguya nüsslini* n. sp.). Arch. Protist., 6: 45-60.

Southwell, T., and B. Prashad. 1918.—On some Indian Myxosporidia. Rec. Ind. Mus., 15: 344-348.

Thélohan, P. 1895.—Recherches sur les Myxosporidies. Bull. sci. France et Belg., 26: 100-394.

EXPERIMENTS WITH STEAM DISINFECTORS IN DESTROYING LICE IN CLOTHING

R. H. Hutchison

U. S. Bureau of Entomology

It has been shown by Nuttall (1918) that "the high standards of efficiency attained by regular disinfectors on the basis of their capability of dealing with bacteria or their heat-resisting spores do not appear necessary for mere louse destruction." This paper deals with the minimum requirements, as regards pressure, time and temperature, for louse destruction only. The question of sterilization does not enter.

The work was done at Camp Mills, L. I., N. Y., in response to a request from the Camp Surgeon to Dr. L. O. Howard, Chairman of the Subcommittee on Entomology, Medical Committee, National Research Council. The request was made at the suggestion of D. L. Van Dine, Captain, Sanitary Corps, who was in charge of the Sanitary Process Plants at Camp Mills, and the work was done in close cooperation with him and with Captain H. L. Gardiner, M. C., Assistant Officer in Charge, in an attempt to answer certain practical problems they had in mind. The principal question was whether the disinfecting process in use at these plants was effective in destroying lice in the clothing, and whether the process would still be effective if the time of treatment were shortened—thus increasing the daily capacity of the plants.

There are two Sanitary Process Plants in operation at Camp Mills. One, referred to as Plant No. 1, is built largely on plans and specifications from Major Harry Plotz (1919) of the Surgeon General's Office. The other, referred to as Plant No. 2, is "home made," so to speak. The nucleus of this plant was an old bath house, and the present establishment is the result of a process of addition and modification dictated by practical experience. Each plant consists essentially of

(1) Disrobing room. Here the men place blankets, overcoats and all other articles of clothing, except leather and rubber goods, in a barracks bag which is issued to them at the entrance. The bags are tied and tagged and placed in the carrier of the sterilizer.

(2) Bath; after a medical inspection the men pass into a bathroom where they are first painted with a kerosene emulsion, then pass to the showers. Here the amount of hot and cold water is not restricted, and the soap supply is liberal, and the men have time for a thorough bath. Those who, on inspection, are found infested with crab lice,

Phthirius pubis, are turned aside to a barber shop where axillary and pubic regions are shaved, after which they pass on into the bathroom.

(3) Drying and dressing room. Here the men dry themselves and are issued bath robes while waiting to receive their clothing from the sterilizers.

(4) Sterilizers. These are the essential factors in the destruction of lice in the clothing. At Plant No. 1 there is installed a large stationary sterilizer which measures about 18½ feet long and 5 feet in diameter. At Plant No. 2 there are twenty portable steam disinfectors arranged in two rows of ten each on opposite sides of a large shed. Each disinfector is rectangular and measures 30 by 42 by 84 inches.

The capacity of Plant No. 1 (February and March, 1919) was seventy men every 40 minutes; of Plant No. 2, 100 men every 40 minutes. The experiments reported below bear upon the question of the efficiency of these disinfectors.

SOURCE OF MATERIAL AND METHOD USED

The species dealt with is the body or clothing louse, *Pediculus humanus,* var. *corporis.* The stock lice were from material which had been reared through several generations during the preceding six months on a healthy individual. This stock was taken from Washington to Camp Mills by the writer. Volunteers were called for from the enlisted personnel of the Medical Detachment to act as hosts for the lice. Two men responded and were assigned by Captain Van Dine to the duty of feeding the lice and rendering other assistance in the experiments. The lice were kept in small pill boxes. These boxes are prepared by punching holes in both top and bottom and covering these openings with chiffon. Each box contained from 50 to 100 lice, together with a piece of cloth to which the lice cling, and to which they attach their eggs. For the purpose of feeding, these boxes were applied to the arm of the volunteers and held in place by means of elastic bands. This was done twice daily, and in the intervals between feedings, the boxes were kept in an electrically heated incubator at a constant temperature of 30° C. Every second day the lice were transferred to clean bits of cloth and returned to the boxes. The eggs or nits on the old piece of cloth, from which the lice had been removed, were counted, and each lot of eggs was put in a separate box. These were labeled and kept in the incubator. In the experiments these eggs were used as the test materials, on the assumption that any means which will destroy the nits will also destroy the active stages of lice, it being a matter of common experience that the nits are the more resistant. When the eggs were subjected to tests they were removed from the boxes and put in the pocket of a coat, blouse or shirt or inside a woolen

sock, thus approximating the conditions of natural infestation. In most cases a woolen sock was used, the bit of cloth with nits attached was put in the toe of the sock, and then a maximum registering thermometer was put in so that the bulb was near the eggs. The sock was then tagged and placed in the roll of clothing of some one of the men who were "going thru the mill" at the time.

The treatment to which the clothing is subjected consists of a preliminary vacuum, 10 inches, for 5 minutes; steam, 15 pounds for 15 minutes, reckoned from time steam is turned on; drying vacuum, 10 inches for 10 minutes.

Steam under 15 pounds pressure gives a theoretical temperature of about 250° F. (121° C.), and on several occasions maximum thermometers gave actual readings of 245° F. (118° C.). This temperature is developed only in the superficial layers of the bags as a rule. The temperature in the center of a mass of goods in a bag seldom goes so high, and then only in bags lightly and loosely packed and not subjected to pressure from weight of other bags above or alongside them. Whether a temperature sufficient to kill is developed in the center of a well packed bag depends on factors influencing the penetration of steam. Some of the factors are:

(1). The manner of packing the bags. The usual method was to spread out the three blankets, folded once lengthwise, on the floor, then the overcoat, blouse and other articles of clothing were arranged on top of these and the whole rolled up and put in the bag. There was, however, a great deal of variation in the way the roll was made up.

(2). The location of the bag in the load. It is obvious that those bags in the center with other bags pressed against them on all sides are more difficult to penetrate than those on the sides or top of the load.

(3). The size of the load. For every carrier there is a certain load beyond which more compression is necessary, which renders it most difficult for steam to penerate within the time limit.

(4) Treatment: Preliminary vacuum; number of inches. Steam under pressure; pounds and time.

(5). The nature of the goods treated, whether wool or cotton, closely or loosely woven.

(6). Moisture and temperature conditions of the goods when placed in the sterilizer.

EXPERIMENTAL DATA

The principal question as to whether the daily capacity of the plants could be increased by shortening the period of treatment of each lot was answered in the negative.

EXPERIMENT 1.—A load of twenty-five barracks bags filled with bath robes was given a very short treatment as follows: Preliminary vacuum, 5 minutes, 10 inches; steam, 5 minutes, 14 pounds reached; drying vacuum, 10 inches, 10 minutes. Lots of eggs and maximum thermometers were placed in center of two of the bags, one of which was located in the center of the load and one on top. In neither case were the eggs killed.

The two following experiments in which the period of exposure to steam was 10 minutes were done at the large sterilizer at Plant No. 1.

EXPERIMENT 2.—Bits of cloth with the nits attached were put in pockets of each of three wool blouses. A maximum registering thermometer was rolled up in each blouse in such a way that the bulb was in close proximity to the spot where the eggs were located. Each blouse was then put in center of a barracks bag filled with bath robes. These two bags were placed in the carrier with twenty-five other bags filled with bath robes.

Results

Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	165	5-7	34.5 C.	116	70.3
B	170	5-7	99.5 C.	0	0
C	158	3-5	77 C.	0	0
Control lot	75	5-7	30 C.*	53	70.6

* Incubator.

Location of Bags in Load.—Bag A was placed in the center of the load. Bags B and C were placed on top.

Treatment.—Preliminary vacuum, 10 inches, 5 minutes; steam, 15-18 pounds, 10 minutes (18 pounds reached at end of 10 minute period); drying vacuum, 10 inches, 10 minutes.

EXPERIMENT 3.—Twenty-five barracks bags were filled with bath robes, and in two of these the test materials were placed. The eggs were put in pockets of a wool blouse. A self-registering thermometer was rolled up in the blouse so that the bulb was near the eggs. The blouse roll was then rolled in four bath robes — thus approximating the conditions of a blouse rolled inside three blankets. The roll above described was put in a bag in which three bath robes had been stuffed in the bottom. The bag was then filled up with other bath robes and tied and tagged.

Results

Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	137	2-4	58 C.	94	68.6
B	120	2-4	36 C.	81	67.5
Control lot	105	2-4	30 C.*	84	80.0

* Incubator.

Location of Bags in the Load.—Bag A was placed in the center of the load, with other bags below, above and stacked at the ends. Bag B was placed on top of the load.

Treatment.—Preliminary vacuum, 5 minutes, 10 inches (reached in 3 minutes); steam, 10 minutes, 15 pounds (reached in 3½ minutes, held 6½ minutes); drying vacuum, 10 minutes, 10 inches.

The peculiar case of a higher temperature recorded in the bag in the center of the load than in that one fully exposed on top, can only be explained on the assumption that some difference in the way the bags were packed interfered with penetration of the steam.

The results of these two trials with the 10 minute period for steam show three out of five cases in which eggs were not killed. Such a high percentage of failures demonstrated that the treatment was of too short duration.

A number of tests were made of the process in regular use at this camp, viz., a preliminary vacuum of 10 inches followed by 15 pounds steam pressure for 15 minutes (reckoned from time steam was turned in), and a drying vacuum of 10 inches for 10 minutes. The results indicated that this period could not be shortened; but on the other hand it was shown that the process was adequate provided certain precautions were observed. These have to do with conditions favoring thorough penetration of the steam, and will be discussed after first describing typical experiments in which all the conditions were favorable.

EXPERIMENT 6.—This test was run with the large sterilizer at Plant No. 1 in the course of regular operations and according to the regular procedure. Two wool socks were prepared by placing in each a thermometer and a bit of cloth with nits attached.

Location in Bags.—A was put in center of a roll consisting of three blankets, two O. D. shirts, two suits of underwear, one overcoat, one blouse, one pair breeches, four pair socks and one towel. B was put in center of a roll of three blankets, one overcoat, one blouse, one pair breeches, extra suit of underwear, wrap leggings and cap.

The underwear which the men were wearing was, after inspection, placed in the mouth of the bag on top the roll.

Location in Load.—Actual load was seventy-three bags, a normal load for this sterilizer. Bag A was placed near the center of the third row of bags, with one below and two above it. Bag B was placed on top of the load.

Treament.—Preliminary vacuum, 10 inches reached in 3 minutes; steam, 15 pounds, 15 minutes, 10 pounds reached in 6 minutes, 15 pounds reached in 8 minutes, held remaining 7 minutes; drying vacuum, 10 inches, reached in 5 minutes and held to end of 9 minutes.

Results Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	222	3-5	104.5 C.	0	0
B	310	3-5	93 C.	0	0
Control lot....	125	3-5	30 C.*	83	66

* Incubator.

The two following experiments conducted at Plant No. 2 in the portable steam disinfectors also demonstrate the efficiency of this treatment when carried out properly.

EXPERIMENT 12.—Two wool socks, each containing a thermometer and cloth with nits were used as before.

Location in Bag.—Sock A was put inside a roll of three blankets. This was placed in the bottom of a barracks bag. On top of this roll the overcoat, breeches, sweater, socks and underwear were crowded. Sock B was put in a

roll of blankets in the center of the bag. Below the roll in the bottom of the bag was the overcoat, blouse and sweater. Above the roll in the top of the bag were underwear, breeches, shirt, socks, cap and wrap leggings.

Location in Load.—Normal load of ten bags was treated. Larger loads for this size sterilizer necessitate undue packing, resulting in difficulty of penetration. Bag A was placed in horizontal position near the center of the carrier. Bag B was put in a vertical positon at one end of the carrier.

Treatment.—Preliminary vacuum, 10 inches, reached in 1 minute; steam, 15 pounds, reached in 4 minutes, held 11 minutes, total 15 minutes; drying vacuum, 10 inches, reached in 3 minutes, held 7 minutes, total 10 minutes.

Results Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	185	7-9	87.5 C.	0	0
B	162	7-9	118 C.	0	0
Control lot....	77	7-9	30 C.	58	75.3

Experiment 14.—This test was carried out in a portable disinfector in which a pressure of 15 pounds was attained. Two wool socks were used with thermometer and nits in each. A was put in a roll of three blankets, two sweaters, one shirt, one blouse. At the sides and top of this roll, after it was in the bag, were stuffed one shirt, two pair socks, two pair breeches, two suits underwear. Bag tightly packed. B was put in roll of three blankets, two suits underwear, two shirts, one sweater, one bouse, one pair breeches. Alongside and on top of this roll were stuffed two pair socks, wrap leggings, cap and underwear. The sterilizer was loaded with eleven bags, the two test bags being near the front end of the carrier.

Treatment.—Preliminary vacuum, 12 inches, reached in 3 minutes; steam, 15 pounds, reached in 5 minutes, held 10 minutes, total 15 minutes; drying vacuum, 10 inches, reached in 5 minutes, held 5 minutes, total 10 minutes.

Results Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	128	6-8	94.5 C.	0	0
B	194	6-8	87 C.	0	0
Control lot....	163	6-8	30 C.	134	79.2

PRECAUTIONS TO BE OBSERVED

The following experimental data bring out some very practical points which have to be kept in mind if the process is to be efficient.

In the first place, less than 15 pounds pressure cannot be used with safety without lengthening the time of treatment. This was brought out in an experiment conducted at Plant No. 2 in a portable disinfector with safety valve adjusted so that only 12 pounds of steam was attained.

Experiment 11.—Two wool socks, each containing a thermometer and nits, were used as before.

Location in Bag.—The same as described for Experiment 12 above.

Location in Load.—There was a load of ten bags put in the sterilizer, and no one bag was completely surrounded by other bags, but had at least one side fully

exposed. One test bag (A) was in a horizontal position near the center of the carrier, and the other (B) was in a vertical position at the rear end of the carrier.

Treatment.—Preliminary vacuum, 10 inches, reached in 2 minutes; steam, 12 pounds, 12 pounds reached in 4 minutes, held 11 minutes, total 15 minutes; drying vacuum, 10 inches, reached in 4 minutes, held 6 minutes, total 10 minutes.

Results

Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	225	7-9	93 C.	0	0
B	287	7-9	51 C.	256	89.2
Control lot	77	7-9	30 C.	58	75.3

Second, the sterilizers must not be overloaded. The tight packing necessary in an overload hinders penetration, especially in those bags in the center of the treated mass. This was shown in the following test:

Experiment 9.—This test was carried out at Plant No. 1 in the large stationary sterilizer during regular operations. Two wool socks were used as before, in each of which was placed a thermometer and a bit of cloth with eggs attached. Each was put in the roll of one of the enlisted men going through at the time.

Location in Bag.—A was put in center of a roll consisting of three blankets, one overcoat, one blouse, two O. D. wool shirts, one pair breeches, one extra suit underwear, one towel, four pair socks, one pair wrap leggings. B was put in another roll made up of practically the same amount of material except that there was one less O. D. shirt, and no towels, nor wrap leggings.

Location in Load.—A was placed in the center of the second row of bags in the carrier, with two bags below and two above it. B had the same position in the fifth row of bags near the other end of the carrier. The carrier was overloaded with eighty-nine bags, which were pressed together and tightly packed.

Treatment.—Preliminary vacuum, 10 inches, reached in 2 minutes, then steam was turned in; steam, 15 pounds, 10 pounds reached in 7 minutes, 15 pounds reached in 9½ minutes, held 5½ minutes, total 15 minutes; drying vacuum, 10 inches, reached in 6 minutes, held 4 minutes, total 10 minutes.

Results

Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	185	5-7	41.5 C.	107	57.8
B	244	3-5	73 C.	0	0
Control lot	118	3-5	30 C.	89	76.7

By way of comparison, the following test shows what a difference the location in a load makes.

Experiment 10.—This test was conducted in much the same manner as in the preceding one. Thermometers and nits were placed in socks and put in roll consisting of three blankets, one overcoat, one blouse, one pair breeches, one extra suit of underwear, socks, leggings and cap. The two rolls in this test were practically alike in amount of material and the method of packing.

Location in Carrier.—Both bags were placed on top the load, which was made up of ninety-three bags tightly packed in the carrier.

Treatment.—Preliminary vacuum, 10 inches, reached after 4 minutes; steam, 15 pounds, reached after 10 minutes, held 5 minutes, total 15 minutes; drying vacuum, 10 inches, reached after 6 minutes, held 4 minutes, total 10 minutes.

Results Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	310	3-5	105 C.	0	0
B	190	3-5	105 C.	0	0
Control lot	116	3-5	30 C.	89	76.7

The two experiments just described were carried out under practically the same conditions except for the location of the bags in the load. The amount of materials was the same in all. The bags were filled in the same way, and the treatment was the same. The difference in the location of the bags in the load had its effect on the results. These bags on top the load, and thus fully exposed, showed a good penetration, a temperature of over 105° C. being developed in the center of both. In those bags in the center of a heavy load (Expt. 9) penetration was rather poor. In one of the two the heat developed in the center of the bag was not sufficient to kill the nits.

A third point of importance to be borne in mind is the necessity of maintaining a full head of steam in the jacket of the sterilizer. With low pressure, the requisite 15 pounds pressure within is reached very slowly. This means failure to kill unless the time of exposure is lengthened, as in the following test.

Experiment 5.—This experiment was carried out at the large sterilizer at Sanitary Process Plant No. 1, in the course of regular operations. In each of two wool socks was placed a piece of cloth with nits attached and a self registering thermometer. Each sock was then placed in the roll of one of the enlisted men going through at the time. Each roll consisted of three blankets, one overcoat, blouse, breeches, two O. D. shirts, extra socks and underwear. The sock with the thermometer and eggs was rolled up in the center of this mass and the roll put in a barracks bag. The underwear worn by the men was, after inspection, placed on top of this roll in the mouth of the bag, which was then tied and tagged in the usual way.

The actual load in this test was ninety-three bags. Not all the bags, however, were completely filled, so that the carrier was not so much overloaded as the figures indicate.

Location of the Test Bags in the Carrier.—Bag A was placed on top of the load. Bag B was placed as near the center of the load as possible. It happened that the underwear worn by the man to whom Bag B belonged was infested with lice so that this offered a test on both the active and the egg stages of the lice.

Results Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	210	4-6	95.5 C.	0	0
B	140	2-4	104.5 C.	0	0
Control lot	155	4-6	30 C.	117	75.4

The lice in the infested underwear in Bag B were found to be dead.

Treatment.—Preliminary vacuum, 10 inches, reached in 3 minutes, held to end of 5 minutes; steam, 15 pounds, reached in 21 minutes, not held; drying vacuum, 10 inches, reached in 10 minutes, not held.

In the above experiment the steam pressure in the boilers was very low. Instead of establishing the required 15 pounds pressure in 7 or 8 minutes or less, and holding it for the remainder of a 15-minute-period, fully 21 minutes were consumed in reaching this pressure. The 15 pounds having been reached, it was released at once. The results are of interest therefore as indicating that penetration can be secured even when the steam pressure is low by a short extension of the time usually given for the application of the steam. It is assumed that the sterilizer is to be operated by one of sufficient intelligence to exercise good judgment in such circumstances. The failure to kill in one of the bags in the following test is to be explained on the ground that the pressure was rather low, requiring 9 minutes to reach the 15 pounds pressure.

Experiment 7.—Two wool socks, containing thermometer and nits, were used as in preceding experiments. Each was placed in the center of a roll of an enlisted man. The two rolls were practically alike as regards the amount of material. Each containing three blankets, one overcoat, one blouse, one pair breeches, extra suit underwear, one O. D. shirt, cap and wrap leggings. There was of course some difference in the way the rolls were made up, as no two men will make up a roll and fill their bags in exactly the same way.

Location in Load.—Actual load was seventy-three bags. Bag A was placed in second row with one bag below and two above. Bag B was placed in fifth row with two bags below and one above.

Treatment.—Preliminary vacuum, 10 inches, reached in 3 minutes; steam, 15 pounds, 15 minutes, 15 pounds reached in 9 minutes and held remainder of the period; drying vacuum, 10 inches, 10 minutes, 10 inches reached in 5 minutes and held 5 minutes.

Results

Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	230	1-3	46.5 C.	217	94.3
B	292	1-3	96.5 C.	0	0
Control lot	122	1-3	30 C.	76	62.3

In Bag A all the contents of the bag was hot except for a very limited area near the center where the bulb of the thermometer happened to be located. In fact, the top part of the steel case protecting the thermometer was too hot to touch on removal while the bulb end was comparatively cool.

Fourth.—It is hardly necessary to point out the value of the preliminary vacuum in assisting the penetration of the steam. However, the following test is included as it brings out the point so clearly.

Experiment 8.—Three wool socks were used, each with a thermometer and a lot of eggs. Each was placed in the center of a roll consisting of three blankets, one overcoat, one blouse, one pair breeches, extra suit of underwear, two O. D. shirts, cap, socks and leggings.

Location in Load.—Actual load was eighty bags. Bag A was placed in second row, two bags below and one above. Bag B was placed in fourth row, two bags below and one above. Bag C was placed in fifth row, two bags below and one above.

Treatment.—Steam, 15 pounds, 15 minutes, 10 pounds reached in 8 minutes, 15 pounds reached in 13 minutes, held at this point for 2 minutes; drying vacuum, 10 inches, 7 minutes, 10 inches reached in 5 minutes.

Results Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	168	5-7	45 C.	111	66
B	220	3-5	37 C.	190	56.3
C	298	3-5	104.5 C.	0	0
Control lot	105	3-5	30 C.	90	85.7

It will be noted that each of the test bags contained about the same amount of material and had the same relative positions in the load. No preliminary vacuum was produced, and failure of penetration is marked in two bags.

Fifth. — The differences in the way the rolls are made up have important influence on penetration. The following test gave results which show failure to kill in one heavy roll tightly packed as compared with success in a light, loose roll.

Experiment 13.—This test was also conducted with the large sterilizer at Plant No. 1. Two wool socks with thermometers and nits in each were put in roll of enlisted men's equipment as follows: A was placed in roll consisting of three blankets, one blouse, one breeches, one O. D. shirt, one cap, two pair socks. B was placed in a roll consisting of three blankets, one overcoat, one blouse, one breeches, two O. D. shirts, one extra suit underwear, four pair socks, one cap, one pair wrap leggings. It will be noted that B was a much heavier roll than A, containing one overcoat, one shirt, one suit underwear, two pair socks and one pair wrap leggings in excess of the material in A. Further, it was kept in compact condition by a web belt which the owner placed around it.

Location in Load.—A was placed in the third row of bags with one bag below and one above. B was placed in the fifth row with one bag below and one above it. The load was a normal one of sixty-nine bags, and the two test bags were placed so as to be about equally exposed.

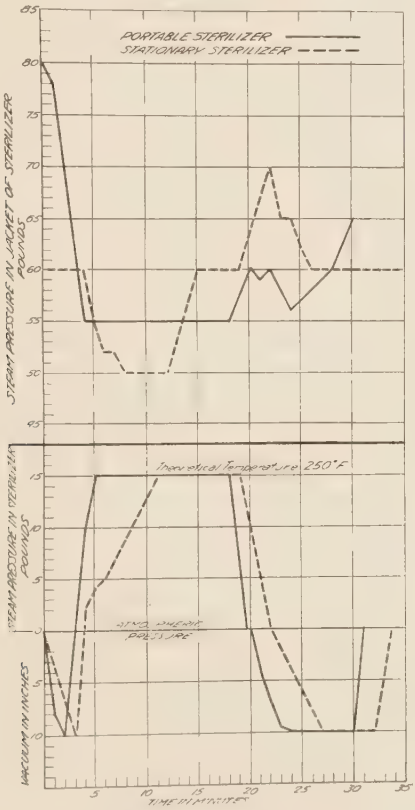
Treatment.—Preliminary vacuum, 10 inches, reached in 3 minutes; steam, 15 pounds, reached in 11 minutes, held 4 minutes, total 15 minutes; drying vacuum, 10 inches, reached in 5 minutes, held 5 minutes, total 10 minutes.

Results Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	210	5-7	85 C.	0	0
B	260	5-7	34 C.	244	94
Control lot	74	5-7	30 C.	46	62.1

In this experiment the difference between the two bags was in the amount of material they contained, and the fact that Roll B was kept tight by a belt. Location in the load and the treatment were the same. The results show that the penetration of the light roll (Bag A) was sufficient to kill all the nits, while in the heavy roll the heat failed to reach the center and none of the nits were killed. Bag B of course contains the usual amount of material which is ordinarily treated with success. The failure to kill was due to making the roll too tight.

A COMPARISON OF THE EFFICIENCY OF THE LARGE STATIONARY STERILIZER AND THE SMALL PORTABLE STEAM DISINFECTOR

A number of records were made of the operation of the sterilizers, noting minute by minute the pressure changes both in the sterilizers and in the jacket. Two of these records, selected because they show the usual changes when the sterilizers are operating well, have been charted (Fig. 1). It will be noted from this chart that the small portable sterilizers do the work more quickly than does the larger stationary



Comparison of the pressure changes in the large stationary and small portable disinfectors during normal operation.

one. The large sterilizer develops the 10 inch vacuum more slowly. It is also much slower in developing the 15 pounds steam pressure, and this is the most serious difference as regards practical results. Records show that the 15 pounds pressure is obtained in the portable sterilizer within 3 to 5 minutes from the time the steam is turned in. Reckoning the 15 minute period from the time the steam is turned on, the contents of the sterilizer is thus exposed to the 15 pounds pressure for 10 to 12 minutes.

In the large stationary sterilizer, the records show that 4 to 11 minutes are required to produce the 15 pounds pressure, thus leaving only 4 to 11 minutes for the application of the pressure.

In every test with the portable sterilizer, the 15 minute period of steam at pressure of 15 pounds was efficient in producing a killing temperature (75° C. and above) in the center of barracks bags packed in the usual way. In one roll, the goods were rolled very tightly, and the man strapped his belt around the roll and kept it tight. On removal from the sterilizer there was one cool spot in the roll under the thick padding of the shoulders of the overcoat. With this exception, which was caused by unusual conditions, we have never found failure of penetration when the portable sterilizers were working with 15 pounds pressure. A 12 pound pressure, however, is not always efficient.

In tests with the large stationary sterilizer, it was found that in three out of ten cases, killing temperatures were not produced in the center of the bags. All of these cases were bags in the center of a large load in the carriers.

It is the writer's opinion that the accompanying chart shows the probable explanation of this difference in efficiency. The solid line, representing operation of the portable sterilizer, shows that, after the 10 inch vacuum was produced, 15 pounds steam was developed within 3 minutes after the steam was turned on, and held for 12 minutes. The broken line, representing action of the large sterilizer, shows that 8 minutes were necessary to raise the pressure to 15 pounds, leaving only 7 minutes during which the goods were exposed.

SHRINKAGE TESTS

Two tests were made to determine the amount of shrinkage resulting from the sterilization process now in use. They were both done in one of the portable sterilizers at Sanitary Process Plant No. 2.

In the first test, two wool blouses, one new and one a re-issue, were folded up with a thermometer in the folds, and put into a barracks bag. This bag contained nothing but the blouses, and was placed on top of a load of eleven bags in the sterilizer. The object was to subject them to the full effect of the heat. They were subjected to the following treatment:

Preliminary vacuum, 10 inches, reached in 2 minutes; steam, 15 pounds, 15 pounds reached in 6 minutes, held 9 minutes, total 15 minutes; drying vaccum, 10 inches, reached in 3 minutes, held 7 minutes, total 10 minutes.

The temperature recorded within the folds was 246° F., which is within 4 degrees of the temperature theoretically developed at 15 pounds pressure.

The blouses were shaken out until cool and dry.

	Measurements	Before Treatment Inches	After Treatment Inches
A	Between shoulders	16½	16¾
	Length of back	29¾	29¾
	Right sleeve, inner seam	19	18¾
	Right sleeve, outer seam	25	24½
B	Between shoulders	16¼	16
	Length of back	29½	29½
	Right sleeve, inner seam	18½	18½
	Right sleeve, outer seam	25	25¼

In the second test, an overcoat, blouse and breeches were rolled up with a thermometer in the center and placed in a barracks bag. Another blouse and breeches were put in a second bag. These two bags were put in sterilizer without any other load. All the clothing in this test was now and pressed. They were treated as follows:

Preliminary vacuum, 10 inches, reached in 2½ minutes; steam, 15 pounds, reached in 2 minutes, held 13 minutes, total 15 minutes; drying vacuum, 10 inches, reached in 2 minutes, held 8 minutes, total 10 minutes.

The temperature recorded in the center of the larger roll was 220° F. It was doubtless higher at the outside of the roll, probably most of the goods were exposed to 245° F.

The clothing was shaken out and measured a second time after they were cool and dry, with following results.

	Measurements	Before Treatment Inches	After Treatment Inches
Overcoat.	Neck	19	18¾
	Length of back	41	40¾
	Right sleeve, outer seam	25	24¾
	Right sleeve, inner seam	19	18½
Blouse A.	Between shoulders	15	15
	Length of back	29¼	29
	Right sleeve, outer seam	25	25
	Right sleeve, inner seam	19¾	19½
Breeches B.	Waist	30	29½
	Left inner seam	26	26
	Left outer seam	38¾	38¼

The two following garments were folded loosely in another bag and probably were subjected throughout to a temperature of about 245 F.

Blouse C.	Between shoulders	15½	15½
	Length of back	28½	28¼
	Right sleeve, outer seam	24¾	24¼
	Right sleeve, inner seam	19¾	19
Breeches D.	Waist	31	31¼
	Left inner seam	26	26½
	Left outer seam	38¾	38½

The results show three measurements one-quarter inch longer after treatment; eight measurements remain the same, and fifteen measurements indicate slight shrinkage. The shrinkage averages only about one-tenth of one per cent. Allowance must be made for some variation in the manner of measuring, and also for the fact that the goods were not pressed after treatment before final measurements.

The above measurements are few in number. Nevertheless, it is safe to say that the sterilizing process as employed at this camp, causes very little if any shrinkage. There is no doubt, of course, that longer exposures to steam under pressure will cause more serious shrinkage. These results agree very well with those of Fulton and Staniford (1918).

CONCLUSIONS

If the penetration of steam is sufficient to produce a temperature of 75° C. (167° F.) in the center of a barracks bag (or other load of infected goods) all eggs and active stages of body lice will be destroyed. This conclusion, based on the above practical tests, agrees very well with laboratory experiments on fatal temperatures. Nuttall (1918) has shown that nits are killed in one minute at 70° C. in dry heat, and in 10 seconds at 70° C. moist heat.

If the disinfectors are operated efficiently on the time schedule now employed (viz., a 10 inch preliminary vacuum; 15 pounds steam pressure for 15 minutes, reckoned from the time the steam is turned on; followed by a 10 inch drying vacuum) the requisite temperature (75° C.) is attained in every case. By efficient operation is meant (1) the maintenance of a full head of steam so that the 15 pounds pressure in the disinfector is produced within 5 minutes, thus allowing at least 10 minutes for exposure; (2) overloading must be guarded against; (3) the individual bundles must not be rolled too tightly.

Little if any shrinkage of woolen goods is caused by this treatment. There is, of course, some wrinkling, but these wrinkles are not permanent but may be remedied by pressing.

The writer wishes to express his appreciation of the courtesy of the authorities of Camp Mills in permitting him to do this work in the camp, and especially to Lieut.-Col. James F. Edwards, Camp Surgeon, and Major Elmer Jackson, Assistant Camp Surgeon, for their active interest and help, and to the Surgeon-General's Office, War Department. for permission to publish the results.

Papers Cited

Fulton, D., and Staniford, K. J. 1918.—The Sterilization of Woolen Blankets and Uniforms. Jour. Am. Med. Assn., 71: 823.

Nuttall, G. H. F. 1918.—Combating Lousiness among Soldiers and Civilians. Parasitol., 10: 411-588.

Plotz, H. 1919.—The Importance of the Louse Problem. Jour. Am. Med. Assn., 72: 324-326.

TWO NEW PROTEOCEPHALIDAE*

Ernest Carroll Faust
Union Medical College, Peking, China

The known Proteocephalidae have been the subject of an adequate study by La Rue (1914). The present paper treats of two new species belonging to the genus Proteocephalus, collected by the writer from the Bitter Root Valley, Montana, in 1915 and 1916. The host of one species, *Ptychocheilus oregonensis* (Richardson), is a cyprinid, while that of the other, *Coregonus williamsoni* (Girard), is a salmonid. In addition to the fact that these parasites have certain unique characters that readily distinguish them from described species, is the record of new hosts for the genus and the location of these worms in a new geographical area.

Proteocephalus ptychocheilus nov. spec.

Host: *Ptychocheilus oregonensis* (Richardson).
Locality: Carlton, Montana.
Date of Collection: April 12, 1915.

Small to medium-sized cestodes, reaching a maximum length of 200 mm. and a maximum breadth of 1.45 mm. Scolex about 1 mm. broad, neck practically as broad. Median fifth sucker lacking; marginal four suckers about 333μ in outer diameter, deep. Proglottids distinct from region about 5 mm. distal to neck. Anterior proglottids two to three times as broad as long. Maturing proglottids longer than broad. Ripe proglottids almost twice as broad as long.

Genital pore lateral, slightly anterior to middle of proglottid, in a shallow depression, sometimes on one side, sometimes on the other. Cirrus pouch constricted in outermost third, dilated in inner two-thirds. Ductus ejaculatorius coiled somewhat in inner dilated region of cirrus pouch; surrounded by prostate glands. Vas deferens consisting of tightly coiled tubule, especially massed in mid-plane of proglottid, with coil extending distad two-thirds distance toward ootype. Testes about 60, irregularly distributed in more than one plane, each about 80 to 110μ in diameter. Vagina opening proximal to cirrus pouch. Vagina traversing vas deferens obliquely. Sphincter vaginae weak, if present. Receptaculum seminalis consisting of a slightly dilated proximal portion of vagina. Vitellaria composed of small follicles, closely crowded together in lateral field just inside longitudinal muscle layers. Ovaries irregular, sacculate. Uterus consisting of six pairs of lateral pouches and a median ventral pocket. Eggs with three membranes, 19.4 by 23μ in diameter of outer membrane.

The specimens on which this study was made consisted of four worms secured from the stomach of a single half-grown female squawfish or chappaul, *Ptychocheilus oregonensis* (Rich.), taken from the

* Contributions from the Zoological Laboratory of the University of Illinois, No. 146.

Bitter Root Valley, at Carlton, Montana, April 12, 1915. The worms were found free in the stomach along with a considerable amount of foodstuff. This host was infected also with *Holostomulum ptychocheilus* Faust.

The scolex of *Proteocephalus ptychocheilus* shows a blunt conical prominence with no indication of a fifth sucker. The four marginal suckers are superficially inconspicuous. Their general outline is spherical, while the long axis of the oval cup-like depression is at right angles to the surface of the cone (Fig. 1). The neck is hardly less broad than the head. Some little distance posterior to the head the worm becomes distinctly attenuated. In the region of maturing proglottids the segments are longer than broad (Fig. 2). Toward the distal end of the chain the proglottids assume a shape about one and two thirds as broad as long. In transection these proglottids are elongate oval.

The mature segments show all of the important sex organs. The genital pore is lateral, slightly proximal to the middle of the proglottid. It is situated in a slight depression; into it open cirrus pouch and vagina. The cirrus pouch is attenuate in its outer third. In this region the wiry cirrus organ is found. Internal to the band of the longitudinal muscles the cirrus pouch enlarges into a sacculate organ which is convexed ventrad. The ductus ejaculatorius works a sinuous course through the inner two-thirds of the contracted organ. The cirrus pouch as a whole occupies a region approximately one-third the width of the proglottid. It has a maximum width of 0.42 mm., and a diameter of 0.14 mm. From the region of the longitudinal muscle band to the innermost region of the pouch the ductus ejaculatorius is surrounded by a limited number of prostate glands. The vas deferens merges into the cirrus pouch imperceptibly. It is directed toward the middle field of the proglottid (Fig. 4), where a great mass of coils is found. Before the duct reaches the midpoint of the segment it turns distad and continues a sinuous course to a region somewhat proximal to the ootype. Here, in the distal third of the proglottid, it receives the vasa efferentia. The testes consist of about 60 oval bodies, located in more than one plane, mostly in the dorsal half of the worm (Fig. 4). They range in size from 80 to 110μ in diameter. Their connecting efferent ducts have not been observed.

The female organs have been observed as follows: The ovaries are a pair of glands in the distal portion of the proglottid, sacculate in outline, extending from the outer border of the vitellaria to the ootype. The organs are constricted internally so that a distinct T is formed with the oviduct junction. Soon after the oviduct emerges from the region of the ovary, it is surrounded by a definite sphincter (Fig. 5, *oc*),

usually designated as the oocapt. It measures about 38μ in cross section. From the oocapt the oviduct has a sinuous course. Toward the ootype, a little distance dorsal to this organ, it is joined by the vagina. The outermost limit of this organ is at the genital pore proximal to the cirrus pouch (Fig. 3). It runs inward, paralleling that organ until it reaches the mass of coils of the vas deferens. Here it crosses under the pouch obliquely toward the midplane, where it is directed distad, and, after a few cramped sinuous bends, runs directly toward the ootype. In this vicinity it joins the oviduct. There is no distinct receptaculum seminalis. Perhaps the dilated proximal region of the vagina, just before it enters the oviduct, functions as such an organ.

The vitellaria are small, numerous follicles, closely compacted into a pair of cords, each within the lateral curve of the longitudinal muscle area. In the region of the ovaries transverse collecting ducts of considerable size converge dorsal to the ootype and the common vitelline duct resulting courses ventrad to join the common oviduct-vagina before the latter runs into the ootype. The ootype is surrounded by a spherical mass of closely crowded gland cells, with a gross diameter of about 230μ.

The uterus emerges from the ootype as a tube of narrow bore (Fig. 5, *u*). After a considerable amount of coiling it runs proximad on the ventral side of the proglottid. It gives rise to six pairs of lateral pouches and a single ventral pouch, the latter in the plane of the cirrus sac (Fig. 4, *up*). This constitutes the original outlet through which the eggs gain access to the outside. The outermost membrane of the subspherical egg measures 19.4 by 23μ. The middle membrane has an average diameter of about 17μ. The primary membrane measures 13μ in diameter. The egg is filled with yolk material.

Proteocephalus ptychocheilus is most nearly related to *P. esocis* (Schneider). However, size differences, structure and shape of cirrus pouch and vas deferens, number and size of testes, amount of vitellaria and details of structure in the vicinity of the ootype, all serve to distinguish the present species as new.

Proteocephalus laruei nov. spec.

Host: *Coregonus williamsoni* (Girard).
Locality: Fort Missoula, Montana.
Dates of Collection: Oct. 25, 1915; Feb. 18, 1916.

Small to medium-sized Proteocephalids, reaching a length of 100 to 120 mm., and a maximum breadth of 1.62 mm. Head and neck not observed. Maturing proglottids longer than broad. Ripe proglottids about one and one-fourth times as broad as long. Segments distinct.

Genital pore lateral, proximal to middle of proglottid. Cirrus pouch elongate, biconvex, extending mesad one-third the width of the proglottid. Cirrus large, muscular. Ductus ejaculatorius bending back on itself at least once

within cirrus pouch. Vas deferens enlarges distad to enclose inner end of cirrus pouch. Coils of vas deferens comparatively few, in middle of the proglottid. Testes 40 to 50, in two planes, each testis 70 to 100μ in transection. Vagina an attenuate tube proximal to cirrus pouch, describing a broad bow under the latter organ (Fig. 7) toward the ootype. Ovaries two, large, irregular. Vitellaria sparse, large, inside main longitudinal muscles. Uterus composed of nine lateral pouches; no ventral pouch observed. Eggs with three membranes, averaging 50 by 42μ in section.

The specimens from which this study was made were secured from two infections of *Coregonus williamsoni* (Girard), taken from the Bitter Root River at Fort Missoula, Mont., Oct. 25, 1915, and Feb. 8, 1916. In the first infection one specimen was found in the mid-intestine of the host. In the second infection five specimens were found attached to the wall of the intestine. In none of the specimens were the heads secured. The absence of the head and neck region makes the structure of the four suckers and the presence or absence of a fifth sucker a matter of conjecture. However, details of the mature and ripe proglottids identify this species as new.

The genital pore is marginal, irregularly alternating right and left. It comes almost to the surface of the marginal line, but at times shows a slight depression. The cirrus pouch is distal and slightly ventral to the vagina. The cirrus and ductus ejaculatorius are both short, so that the cirrus pouch extends mesad only about one fourth of the proglottid distance. The entire lumen is surrounded by a large number of closely grouped prostate glands. The inner end of the cirrus pouch projects into the enlarged outer portion of the vas deferens. It is reflexed acutely and reinforced by integument (Fig. 9). The vas deferens becomes slightly smaller as it courses toward the midfield of the proglottid. In this region it coils several times and here receives the vasa efferentia. The testes are distributed in two planes, mostly lateral to the uterine pockets. They number 40 to 50 and vary in cross section diameter from 70 to 100μ. The vasa efferentia have not been observed.

The vagina arises at the genital pore as a tube of small caliber. It runs mesad on the proximal side of the cirrus pouch and crosses under the vas deferens near the juncture of that organ with the cirrus pouch. It bends distad as it approaches the midfield and continues to the ootype (Fig. 7). The ovaries lie an appreciable distance from the distal margin of the proglottid. They are large irregular bodies, which may or may not become attenuated toward the midfield.

The vitelline follicles are large, sparsely scattered bodies, numbering about twenty on a side. They are often entirely lacking in sections of 8 to 10μ (Fig. 9). Their transverse collecting ducts unite to form a common vitelline duct which runs ventrad toward the ootype (Fig. 8).

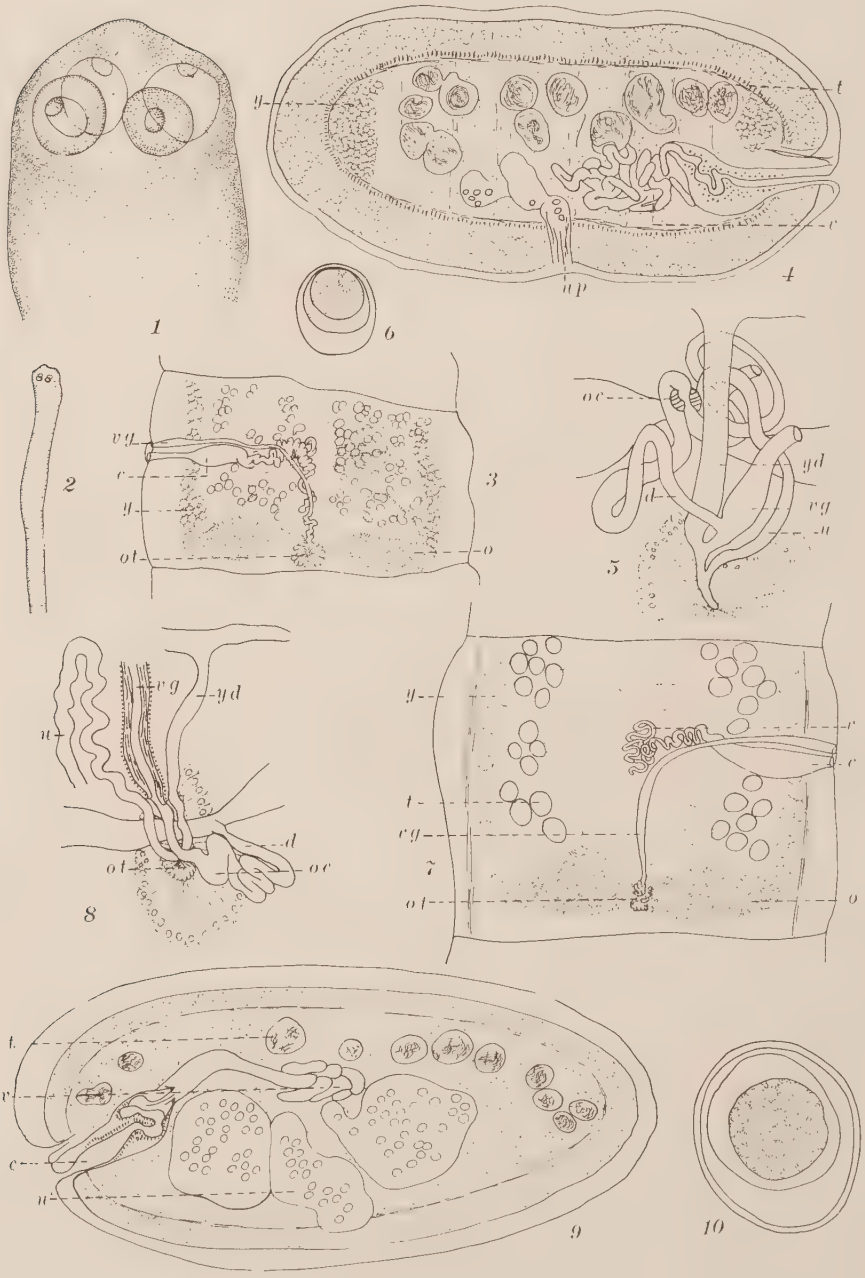

y
t
c
ap
4
1
6
2
vg
c
y
ot
o
3
oc
yd
d
vg
u
5
vg
yd
u
d
ot
oc
8
y
c
c
t
vg
7
ot
o
t
v
c
u
9
10

PLATE VI

The ootype with its surrounding glands is a pyriform organ. The gland cells consist of two types: small, densely crowded ones immediately surrounding the ootype, and larger ones enveloping the whole. From the common limb of the two ovaries arises the oviduct which coils on itself several times, and, after passing through a muscular enlargement, the oocapt (*oc*), is joined by the vagina. Thence it runs into the ootype. The proximal end of the vagina consists of a thick wall well supplied with inner longitudinal and outer transverse muscles (*vg*), surrounding a small lumen. Between the junction of the vagina and the oviduct and the ootype the vitelline duct merges into the common duct, so that the combined products are emptied into the ootype through one duct. The uterus emerges from the ootype as a small sinuous tube. Proceeding proximad it enlarges to form nine irregular pockets on each side of the midline. No ventral pocket for the emission of the eggs from the uterus has been found. The eggs are enveloped in three membranes, an outer one of a heavy consistency, with a diameter of 42 to 50μ, a middle one some 39μ in transection, and an innermost one of 25μ measurement (Fig. 10). The egg is filled with a dense mass of vitelline granules.

Proteocephalus laruei bears some resemblance to *P. cernuae* (Gmelin) La Rue (1914). It differs in the distribution of the testes, in the number of the vitellaria, in the details of the cirrus pouch and in organs around the ootype. In addition, the structure of the egg membranes is different.

Reference Cited

La Rue, G. R. 1914.—A Revision of the Cestode Family Proteocephalidae. Ill. Biol. Monogr., 1: 1-350, 16 pl.

Description of Plate

Proteocephalus ptychocheilus. 1. Enlarged view of head of worm, showing scolex, suckers and neck, × 34. 2. Habit sketch of anterior end, × 4. 3. View of mature proglottid, × 34. 4. Cross section of proglottid in region of cirrus pouch, × 54. 5. Diagram of organs in vicinity of ootype, × 105. 6. Egg, × 540.

Proteocephalus laruei. 7. Mature proglottid, × 34. 8. Detail of organs in region of ootype, × 105. 9. Cross section of proglottid in region of cirrus pouch, × 54. 10. Egg, × 540.

Abbreviations Used

c cirrus pouch.	*u* uterus
d oviduct	*v* vas deferens
o ovary	*vg* vagina
oc oocapt	*up* ventral uterus pocket
ot ootype	*y* vitellaria
t testis	*yd* vitelline duct

ON THE RESISTANCE TO DESICCATION OF THE INTERMEDIATE HOST OF SCHISTOSOMA JAPONICUM KATSURADA

WILLIAM W. CORT
School of Hygiene and Public Health, Johns Hopkins University

In an earlier paper (Cort, 1919: 489) I noted that specimens of *Blanfordia nosophora* (Robson), the intermediate host of the Japanese blood fluke, *Schistosoma japonicum,* which had been shipped from Japan to California in a dried condition, became active when placed in water. This resistance to drying is possible because *Blanfordia nosophora* is an operculate snail and is able to close the opening of its shell with its operculum. In this connection it seemed important to determine the degree of resistance to desiccation of this species of snail. The practical significance of definite data on this point is evident, since the destruction of the snail intermediate host is a promising line of attack in the control of any trematode parasite of man. Material for taking up of this problem became available when on June 23, 1919, I received through the kindness of Dr. S. Yoshida, of the Osaka Medical College, a large number of dried specimens of *Blanfordia nosophora* which had been collected in Japan May 28. Examination showed that over 85 per cent. of these snails were still alive, and that about 4 per cent. of the living snails were infected with the cercariae of *S. japonicum.*

The method of carrying on the experiments involved in this problem was as follows: The dried snails were carefully counted out in lots of one hundred and examined at intervals ranging from one to several days (Table 1). Each hundred snails at the time set for examination was placed in a beaker of water. After about fifteen minutes the snails would begin to emerge from their shells, and inside of two hours almost all of the living ones would be active. These snails were then crushed in watch glasses and examined carefully with a microscope for schistosome cercariae. The snails which had shown no signs of life after two or three hours were kept over night in the water. If any more proved to be alive they were also examined microscopically. In part of the examinations made during the first two weeks of the work, that is up to July (Table 2), the snails which showed no signs of life were also crushed and examined with a microscope. They were found without exception to be dead.

In my experiments the attempt was made to answer the following questions: First, how long can specimens of *Blanfordia nosophora* live in the dried condition? And second, do snails infected with the cercariae of *S. japonicum* show less resistance to desiccation than uninfected ones?

TABLE 1.—DATA SHOWING RESISTANCE TO DESICCATION OF BLANFORDIA NOSOPHORA

Date of Examination	Number of Days after Snails Had Been Dried	Number of Snails Examined	Number of Living Snails	Number of Living Snails Infected with Cercariae of S. japonicum
June 25	28	100	86	4
June 26	29	100	87	3
June 26	29	100	82	1
June 27	30	100	91	4
June 27	30	100	90	1
July 5	38	100	80	1
July 5	38	100	83	2
July 6	39	100	76	1
July 7	40	100	81	1
July 7	40	100	77	1
July 9	42	100	76	2
July 11	44	100	71	0
July 14	47	100	62	0
August 7	71	100	12	1
August 7	71	100	8	0
August 12	76	100	2	0
August 22	86	100	0	0
August 24	88	100	0	0

The data given in Table 1 shows that the length of life of *Blanfordia nosophora* in the dried conditions is comparatively short. From the fourth to the seventh week after the snails had been taken out of the water, June 25 to July 14, there was a distinct decrease in the number of living individuals. After ten weeks, August 7, only a small percentage of the snails were found to be alive, and finally in less than three months, August 22, all individuals were found to be dead. This last result was corroborated by data from two other entirely distinct batches of material of *Blanfordia nosophora*. A number of specimens were set aside from snails which were taken out of the water in Japan on March 27. On August 7, when this material was examined, all of the snails were found to be dead. Further material comprising about a pint of dried snails was brought from Japan by Dr. S. Yoshida. All were found to be dead when they were placed in water three months after they first became dry.

Table 2 contains seven one hundred lots taken from Table 1 in which microscopical examinations were made of the dead snails, as well as of the living. A large proportion of the dead snails were simply empty shells. Part of them, however, had apparently only recently died, and in some of these it was still possible to detect the infection with the cercariae of *S. japonicum*. Of the 700 snails included

in Table 2, 573 were found to be living and 127 dead. I have no record of how many of these 127 dead snails were merely empty shells. The table shows that 11 of the living snails, or 1.9 per cent., and that 7 of the dead snails, or 5.5 per cent., were infected with cercariae of *S. japonicum*. Since a large number of the dead snails were merely empty shells the proportion of infected snails among those which had died during the course of the experiment was much greater than 5.5 per cent. These figures certainly indicate a more rapid death rate among infected than uninfected snails.

TABLE 2.—DATA ON LOTS IN WHICH BOTH LIVING AND DEAD SNAILS WERE EXAMINED FOR INFECTION WITH THE CERCARIAE OF S. JAPONICUM

Date of Examination	Number of Days after Snails Had Been Dried	Number of Snails Examined	Number of Living Snails	Number of Living Snails Infected	Number of Dead Snails	Number of Dead Snails Infected
June 25	28	100	86	4	14	2
June 27	30	100	90	1	10	1
July 5	38	100	80	1	20	2
July 5	38	100	83	2	17	0
July 6	39	100	76	1	24	2
July 7	40	100	81	1	19	0
July 7	40	100	77	1	23	0

The data (Table 1) also show a distinct reduction in the percentage of infection in the living snails during the course of the experiment. In the first 500 snails examined, June 25 to June 27, the percentage of infection of living snails with the cercariae of *S. japonicum* was 3.44 per cent. In the second 500 snails examined eight days later, July 5 to July 7, the percentage of infection in living snails was only 1.51 per cent. On the other hand a striking case of resistance of an infected individual is shown in the first 100 snails examined on August 7 (Table 1), in which one infected individual was found out of 12 still living after the snails had been in the dried condition for ten weeks.

It was evident, however, that desiccation unfavorably affected the cercariae of *S. japonicum* within the snail host. All cercariae taken from snails which had been dry for a month or more were somewhat shrivelled and inactive. Many of these cercariae became plump and active after being in water for a while. Quite a number of cercariae, however, in each of these infected snails were permanently injured, and in some cases almost all the cercariae in a snail would be rendered entirely inactive. The conclusion can therefore be drawn in regard to the second question proposed, that not only are the infected snails less resistant to desiccation than the uninfected, but that the cercariae are injured and sometimes killed by a length of time in the dried condition that their host is able to resist.

It is perhaps significant to note in this connection certain habits of *Blanfordia nosophora* which are related to its resistance to drying. I have found it difficult to keep snails active in aquaria. They keep constantly climbing out of the water and drying on the glass. They became almost immediately active again when pushed back into the water. This habit of crawling out of the water seems to be related to unfavorableness of environment in the aquaria. In those aquaria in which there was present a considerable amount of food material, as indicated by a slight bacterial film on the surface of the water and in which the water was kept well aerated, very few of the snails crawled above the water. On the other hand, in aquaria containing little food material or in which the water was foul, almost all the living snails would soon be found dry above the surface of the water. It was possible to determine whether a given aquarium offered a favorable environment or not by the number of snails which remained active under the water. It is evident that this habit of going out of the water to escape unfavorable conditions would be of importance in maintaining the numbers of this species of snail in an environment such as the rice fields of Japan, where there are many changes in the level and the condition of the water. It would also make it difficult to destroy the snails in any given body of water, by the introduction of chemicals.

The data obtained from the experiments recorded in this paper have a definite relationship to measures for the control of Japanese schistosomiasis. The snail intermediate host has long been considered a vulnerable point for attack in the life cycle of digenetic trematodes injurious to man. In the fight against the sheep liver fluke, *Fasciola hepatica*, the killing of the snail intermediate host and the destroying of its breeding places by draining infected pastures, has always been the most effective measure of control. Leiper (1915) advocates this same method in the control of schistosomiasis in Egypt. He suggests that all temporary pools or ditches which harbor the intermediate hosts of *Schistosoma haematobium* and *Schistosoma mansoni* be drained. Since these snails are non-operculate and have little resistance to desiccation, Leiper's suggestion would seem to offer an effective method of control. The control of Japanese schistosomiasis in this way will prove more difficult on account of the resistance to drying of *Blanfordia nosophora*. The experiments outlined above show that this resistance is limited and wherever it would be possible to dry up breeding places for more than three months, the snails would be killed. Also even if the length of time of drying could not be carried to the full three months limit, some progress would be made on account of the reduction in numbers of the snails and the death of infected individuals.

CONCLUSIONS

1. The resistance to desiccation of *Blanfordia nosophora,* the intermediate host of the Japanese blood fluke, *Schistosoma japonicum,* is limited to about three months.

2. Desiccation unfavorably affects the cercariae within the snails and infected snails succumb more quickly than uninfected.

3. Individuals of *Blanfordia nosophora* will voluntarily leave the water and become dry under unfavorable conditions.

4. Measures for the control of Japanese schistosomiasis by draining the breeding places of *Blanfordia nosophora,* would be fully effective only if these places were kept dry at least three months.

LITERATURE CITED

Cort, W. W., 1919.—The Cercaria of the Japanese blood fluke, *Schistosoma japonicum* Katsurada. Univ. Calif. Pub. Zool., 18: 485-507.

Leiper, R. T. 1915.—Report of the results of the Bilharzia Mission in Egypt, 1915, II, Prevention and eradication. Jour. Roy. Army Med. Corps, 25: 147-192.

A MOUSE OXYURID, *SYPHACIA OBVELATA*, AS A PARASITE OF MAN*

WILLIAM A. RILEY
University of Minnesota

In December, 1918, the late Dr. A. F. Coutant sent me from Zamboanga, Philippine Islands, a sample of fecal material containing tapeworm segments for identification. The sample was from an American Bohemian child living in Zamboanga. She was one of a family of five, all of whom were heavily infested by the worm in question.

Examination of the material showed the presence of eggs and of fragments of the rat tapeworm of man, *Hymenolepis murina* (*H. nana*). This species, until recently regarded as very rare in man, has been found in the course of the hookworm investigations in the South to be fairly common. Indeed, the prophecy made by Dr. Stiles soon after the commencement of that work, that "*Hymenolepis nana* will be found to be the commonest tapeworm in the United States" has been amply justified. Its minute size and the failure of physicians to make routine feces examinations had resulted in its being very largely overlooked, until the intensive studies of the hookworm campaign incidentally brought it to light. Of the more recent statistics there may be cited the studies of Frey (1915), who found this tapeworm in 32.6 per cent. of the inmates of the Texas State Orphans' Home. This percentage was exceeded only by that of hookworm infestation in the same group of 270 children.

While the tapeworm which had attracted attention thus proved to be one already noted in man, the search through the material led to the finding of eggs and two specimens of an Oxyurid hitherto unreported for man. Since the feces sample had been preserved by adding 6 to 10 per cent. formalin solution it is probable that the actual strength of the diluted solution did not exceed 3 to 4 per cent. Thus the fixation of the worms was imperfect, but careful comparison with specimens and with the detailed descriptions by Seurat (1916) have convinced me that the species under consideration is *Syphacia obvelata* (Fig. 1). This nematode has been until recently classed in the genus *Oxyuris*, but more critical work has resulted in its being separated by Seurat as the type of a new genus *Syphacia*. Like the tapeworms

* Pubished with the approval of the Director as Paper No. 182 of the Journal Series of the Minnesota Agricultural Experiment Station.

present in the same sample, it is a species which is known to occur in rats and mice.

DESCRIPTION

Both of the worms found in the sample were mature females. Unfortunately, one of the specimens was destroyed in laboratory class work before its value was appreciated. The following description, measurements and figure are from the remaining specimen mounted ventral side up in glycerin jelly.

Female (Fig. 2) elongate-fusiform, measuring 3.7 mm. in length by 0.3 maximum thickness. Cuticula finely cross-striate; two small cervical alae. Mouth surrounded by three broad lips. The body terminates in a long tail which measures from the anus to its tip 0.6 mm. About the anus are fragments of the sepia brown fungous growth noted by von Linstow and by Hall as common on the skin of many females of *Syphacia obvelata.*

The club-shaped oesophagus measures 300μ in length to the point where it terminates in a subspherical bulb. Bulb 100μ long. Vulva prominent, situated 100μ caudad of the oesophageal bulb, or 500μ from the anterior end. Excretory pore opening behind the oesophageal bulb, about 250μ in front of the vulva.

Eggs (Figs. 3, 4) are of the typical oxyurid type, asymmetrical, flattened on one side, measuring 125μ by 40μ. The embryo is evident in some of the eggs.

COMPARISON WITH OXYURIS VERMICULARIS

There is a striking difference in size between specimens of *Syphacia obvelata* and those of *Oxyuris vermicularis.* Our specimens of the former from man measure 3.7 mm. Other specimens of the same species from rodent hosts vary within moderate limits, Hall (1916) giving the range for females as 3.5 to 5.7 mm. On the other hand, females of *Oxyuris vermicularis* range from 9 to 12 mm. in length. The males of *Syphacia obvelata* measure from 1 to 1.6 mm. in length, as compared with 2 to 5 mm. for males of *Oxyuris vermicularis.*

Still more striking are the differences between the eggs of the two species, those of *Syphacia obvelata* (Figs. 3, 4) having over twice the length of those of *O. vermicularis* (Fig. 5), and also being more fusiform. The average measurements for those of *Syphacia obvelata* are 125μ by 40μ; for those of *O. vermicularis* 52μ by 24μ. Both are asymmetrical, those of *S. obvelata* being the more strikingly so.

More fundamental differences of structure have led various writers not only to distinguish generically between Syphacia and Oxyuris, but to remove the well-known human parasite *Oxyuris vermicularis* from

the genus Oxyuris. Seurat (1916) established for this species the genus Fusarella, but Railliet and Henry (1916) have shown that this must give way to the older name Enterobius Leach 1853. Thus the pin-worm of man, almost universally known in the medical literature as *Oxyuris vermicularis,* is more correctly designated *Enterobius vermicularis* (L. 1785) Leach 1853. The type of the genus *Oxyuris* is *Oxyuris equi* (Schrank 1788).

INFECTIONS OF MAN BY OXYURIS INCOGNITA

Shortly after this study was begun, there appeared a paper by Kofoid and White (1919) recording the finding of a nematode ovum, apparently undescribed, in 427 cases among approximately 140,000 soldiers examined at Camp Travis, Texas, and of various military units of the Southern Department (Texas, Oklahoma, New Mexico and Arizona).

In as far as there were published data the writers were certainly justified in stating that "this ovum is the largest ovum of intestinal worms encountered in human stools." Their measurements showed its average dimensions as 95μ by 40μ, with a ratio of length to diameter of 2.4:1. It is marked by the asymmetry typical of eggs of the Oxyuridae.

"The infected soldiers were examined for most part within two or three weeks after admission from civil life, hence the infection may be attributed to the region of their previous residence. The distribution has been determined on the basis of the place of enlistment of the infected soldiers. In 30,348 examinations made between July 28 and August 21, 1918, there were 361 cases of infection among troops in Camp Travis. These came from forty-eight states of the Union, with infections in twenty-two states."

Though no adult worms were discovered in these examinations, the writers concluded that the eggs are those of an Oxyuris to which they tentatively give the name *Oxyuris incognita.*

When Kofoid and White's report first came to my attention I thought it probable that we were dealing with the same parasite. This seemed the more possible, since they state that the egg which they found "is extraordinarily variable in size and proportions, its length ranging from 69 to 133 microns and its diameter from 33 to 43." On more careful examination, however, I found that none of the normal eggs from my specimens were as small as the 95μ which they give as the average. This was also true of eggs from available specimens of *Syphacia obvelata* from mice. Hall (1916) gives the length of eggs of this species as 110 to 142μ.

The other common Oxyurid infesting rats and mice in this country is *Oxyuris tetraptera.* The eggs of this species are much smaller than those of *S. obvelata,* averaging about 90μ in length by 36μ in width. It is possible that both of these species are capable of development in man, and that the wide variations in measurements obtained by Kofoid and White were due to mixed infections — a very common condition in the rodent hosts. It seems more probable that *Oxyuris incognita* represents a species as yet unknown except in the egg stage.

SOURCE OF HUMAN INFESTATION

From the available data relative to the case here reported, it is evident that the food of the child and of others of the family had been grossly contaminated by mice or rats. This accounts for the infestation by one of the commonest nematode parasites of these rodents.

Incidentally, it furnishes circumstantial evidence in favor of the view that *Hymenolepis nana* of man and *Hymenolepis murina* of rodents are one and the same species, as has been claimed, on morphological grounds, by various investigators. Grassi has shown, and we have repeatedly verified in experimental work, that *Hymenolepis murina* is able to complete its development in the intestines of a single host from eggs which have been ingested. The embryos develop in the villi of the intestines and the cysticercoids there produced drop into the lumen of the intestines and develop into the adult worms without the necessity of being transferred to another host.

Thus contamination of food by mice may be the cause of both cestode and nematode infestation of man. Why the nematode infestation is not more common is not clear. The failure to recognize it may be a question of the eggs being less abundant in the feces, or of being subject to marked seasonal variations in appearance, as suggested by Kofoid and White for the species with which they were dealing.

References Cited

Frey, J. H. 1915.—Helminthiasis at the Texas State Orphans' Home. Texas State Journal Med., 11: 229-231.

Hall, M. C. 1916.—Nematode parasites of mammals of the orders Rodentia, Lagomorpha and Hyracoidea. Proc. U. S. Nat. Mus., 50: 1-258.

Kofoid, C. A., and White, A. W. 1919.—A new nematode infection of man. Jour. Amer. Med. Assn., 72: 567-569.

Railliet, A., and Henry, A. 1916.—Sur les Oxyurides. C. R. soc. biol. Paris, 79: 113-115.

Seurat, L. G. 1916.—Sur les Oxyures des mammifères. C. R. soc. biol. Paris, 79: 64-68.

PLATE VII

Explanation of Plate

Fig. 1.—*Syphacia obvelata* (Rudolphi 1802) Seurat 1916, from the cecum of the mouse, *Mus musculus*. × 50.

Fig. 2.—*Syphacia obvelata* from a child, Zamboanga, Philippine Islands. The preparation has been unduly compressed, and was twisted at the caudal end. × 50.

Figs. 3 and 4.—Eggs of *Syphacia obvelata*. × 720.

Fig. 5.—Egg of *Oxyuris vermicularis* drawn to the same scale as Figures 3 and 4.

The drawings were made under direction by G. H. Childs. Figure 5 was redrawn from Braun.

OBSERVATIONS ON *DIOCTOPHYME RENALE* IN DOGS

GEORGE B. WISLOCKI

Arthur Tracy Cabot Fellow, Laboratory of Surgical Research
Harvard Medical School

In a series of routine autopsies on 3,200 dogs, a number of specimens of *Dioctophyme renale* (*Eustrongylus gigas*) were encountered. It seems of interest to tabulate the findings in such a large series of animals as regards the sex of the parasites, their length, the number of specimens encountered in a single host, the region in which they were located, the sex of the host, etc.

The autopsies were performed in the city of Washington between May 1 and Dec. 1, 1918, on dogs which came from the District of Columbia, Virginia, Maryland and Pennsylvania. The animals were of all ages, breeds and sizes.

At autopsy the thorax and abdominal cavity of each animal were opened and the viscera removed. In every instance in which a parasite was encountered the lesions were noted and the tissues sectioned.

In the following table the findings are briefly presented:

Sex of Host	Number of Parasites Found	Sex of Parasite	Length of Parasite	Location of Parasite	Condition of Kidneys
Dog 1, Male	One	Female	61 cm.	Peritoneal cavity	Right kidney atrophic and shrunken; imbedded in scar tissue. Left kidney normal
Dog 2, Female	One	Female	73 cm.	Peritoneal cavity	Normal
Dog 3, Female	One	Female	71 cm.	Peritoneal cavity	Normal
Dog 4, Male	One	Female	35 cm.	Peritoneal cavity	Normal
Dog 5, Female	One	Male	30 cm.	Peritoneal cavity	Normal
Dog 6, Female	One	Female	63 cm.	Peritoneal cavity	Normal
Dog 7, Male	Three	Male	25 cm.	Peritoneal cavity	Normal
		Female	61 cm.		
		Female	63 cm.		
Dog 8, Female	One	Female	38 cm.	Peritoneal cavity	Normal
Dog 9, Male	One	Male	28 cm.	Peritoneal cavity	Normal
Dog 10, Female	One	Female	51 cm.	Peritoneal cavity	Normal
Dog 11, Female	Two	Female	68 cm.	Peritoneal cavity	Right kidney atrophic and shrunken; imbedded in scar tissue. Left kidney hypertrophied
		Female	58 cm.		
Dog 12, Male	One	Female	102 cm.	Peritoneal cavity	Normal

Twelve dogs in 3,200 harbored the parasite, a ratio of 1:266, or 0.37 per cent. It will be noted that five hosts were males and seven females. Two animals contained more than one worm. The majority of the invading organisms were females.

The organisms occurred in every instance free in the peritoneal cavity, and only twice could a portal of entry, through a partially destroyed kidney, be surmised.

The lesions of the peritoneal cavity are of interest. Constant trauma to the peritoneum, associated with the escape and absorption of blood, and the irritating effect of the organism's excreta and ova produce a chronic peritonitis. A dirty brown or greenish, odorless exudate of fibrinous character is observed in places clinging tenaciously to the roughened peritoneal surface. The omentum is often matted together with exudate and frequently adheres so intimately to the liver, spleen, pancreas and intestines that it is difficult to disentangle them. The mesenteric, preaortic and mediastinal lymph nodes are enlarged and deeply pigmented. Fibrous scars and adhesions are occasionally noted about the spleen and the liver.

On examining the thickened omentum or peritoneal surfaces microscopically, one finds them covered with a cellular exudate composed of polymorphonuclear leukocytes and mononuclear cells among which numerous ova are embedded. These ova are slowly disintegrating under the action of one or more giant cells by which each of them is surrounded. The cytoplasm of many of the mononuclear cells contains particles of detritus and pigment.

DISCUSSION

Interest attaches to this parasite because of its occasional occurrence in man. Blanchard (1886) reviewed the literature on the subject and found only nine human cases which he considered authentic. These had all been reported from Europe. Stiles (1898) states that up to that time no authentic human case had been reported in the United States. For an enumeration of the human cases and a description of the generic diagnosis, synonymy and other data concerning the parasite reference is made to Stiles. Stitt (1918) states that there seem to be seven authentic and nine doubtful cases of infection in man. The parasite in the human being is usually described as located in the dilated renal pelvis, and it is due to this circumstance that it is often spoken of as the giant kidney worm. In the fatal cases death results from peritonitis and hemorrhage following rupture of the distended kidney.

Balbiani (1870), in a number of experiments, attempted to transmit the infection directly by transferring the ova from one animal to another, but he was unsuccessful. From these experiments he concluded that an intermediate host must exist. He further observed the development of embryos from the ova and noted the fact that they would remain alive for many years in the presence of moisture. It is

thought that part of the life cycle of the organism is passed in fish, since larvae of the genus Dioctophyme have been found in certain species.

Besides in man, the organisms have been described in the dog, wolf, martin, mink, seal and other mammals. Geographically, they have an almost universal distribution.

In the United States and Canada this parasite has been most frequently described in the dog. Complete data on the length, sex of the parasite, etc., has seldom been given in the cases reported. Welch (1890) noted four of these organisms in the body cavity of dogs, and gave the length of one of them as 95 cm. Crowe (1907) mentioned two others, one of which occurred in the peritoneal cavity. He stated that the kidneys were normal and that there were no lesions of the peritoneum.

Riley (1916) found twenty-seven cases reported for the United States and Canada. He stated that in twelve of these the worms were found in the peritoneal cavity, but that in the majority they occurred in the dilated renal pelvis.

Hall (1917), besides reviewing the subject to date, added some observations of his own. His cases, added to Riley's, make a total of thirty-two for the United States and Canada. From some personal communications he later placed the total still higher. Hall stated that in one half of all the cases the organisms occurred in the peritoneal cavity. Stratton (1843) believed that the organisms invade the peritoneal cavity through the fallopian tubes, and Hall found that in nine out of ten cases in which the sex of the host was given, they were females. In my cases, however, only seven out of twelve of the hosts were females, showing that in all probability the fallopian tubes play no essential part in the entry of the parasite into the body cavity. That the parasite enters the peritoneal cavity through the kidney seems probable in those instances in which a ruptured or scarred kidney is associated with its presence. Frequently no lesions of the urinary tract are discoverable. In my series ten out of twelve hosts showed neither macroscopic nor microscopic lesions of the kidneys or ureters, and in these instances there is no clue as to how the organism gained access to the peritoneum. Crowe (1907) mentioned two cases, with the organism located in the peritoneal cavity, in which there were no discoverable lesions of the kidneys.

Sommer (1896), in examining fifty dogs in Washington for parasites, found this parasite in 2 per cent. Hall states that in examining a series of seventy-six dogs in Washington, he found none. In a series

of sixty-seven dogs in Michigan, he found it in two animals, or 3 per cent. The writer, in the present very much larger series, finds an incidence of only 0.37 per cent.

References Cited

Balbiani, G. 1874.—[Remarks] Compt. rend. soc. biol. Paris, 14: 125.

Blanchard, R. 1886.—Nouvelle observation de strongyle geant chez l'homme. Compt. rend. soc. biol. Paris, 3: 379.

Crowe, S. J. 1907.—The Parasites of Baltimore Dogs. Johns Hopkins Hosp. Bull., 18: 464.

Hall, M. C. 1917.—Parasites of the Dog in Michigan. Jour. Amer. Vet. Assoc., n.s. 4: 383.

Riley, W. A. 1916.—The Occurrence of the Giant Nematode *Dioctophyme renale* in the United States and Canada. Journ. Amer. Vet. Assoc., n.s. 2: 801.

Stiles, C. W. 1898.—Notes on Parasites. Med. Record, 53: 469.

Stitt, E. R. 1918.—Practical Bacteriology, Blood Work, and Animal Parasitology. Phila., Pa.

Welch, W. H. 1890.—Remarks and Exhibition of Animal Parasites. Johns Hopkins Hosp. Bull., 1: 72.

SARCOSPORIDIOSIS IN AN EAST INDIAN*

S. T. Darling
Professor of Hygiene, Faculdade de Medicina e Cirurgia de S. Paulo

Reports of cases of sarcosporidiosis of man are extremely rare. There are probably only two other undoubted cases in the literature: the case reported by Baraban and St. Remy in 1894, and the second reported by me in 1909.[1]

The finding of two cases is due probably to a rather persistent search for parasites in a very large postmortem service in the tropics. The infection is probably of little or no pathological importance in man, but as an example of what may be the lodgment of a sporozoon in a biological blind alley it has some interest.

CLINICAL HISTORY

Ali, a mohammedan Malabari, bullock cart driver and estate coolie, 30 years of age. Had come from Ponani, Malabar Coast, British India, two years before his death and had lived in Serembam and Ampang, Federated Malay States, for two years.

He was a patient of the District and General Hospitals, Kuala Lumpur, having been treated for malaria and hookworm infection. He was admitted to our ward[1] in the District Hospital on July 19, 1915, and was under treatment and care until September 28, the day of his death.

He was suffering from severe anemia of a type not uncommon in the Federated Malay States. Subtertian malarial plasmodia were found on admission by Dr. Barber, and he was treated for hookworm by Dr. Hacker and fifty-three hookworms were expelled. The erythrocyte count on the day of admission was 952,000.

His case was of interest to us, for he presented a picture of severe anemia which we believed was due chiefly to longstanding, insufficiently treated malaria. It is unnecessary to go into the details of his clinical course in the ward, for although extremely interesting as an example of untreated malarial cachexia it probably bears no relation to the slight infection by sarcosporidia which was found at the autopsy.

During his life his tongue presented a picture of desquamation and atrophy seen so commonly in the cachectic state following malaria

*Published under the auspices and support of the Government of S. Paulo, and the Rockefeller Foundation.

1. Malaya Board working under auspices of British Colonial Office and Rockefeller Foundation in Federated Malay States.

that is sometimes called sprue. Monilia were detected in scrapings from the tongue, and his stools were frothy, but there was no invasion of the tissues of the gastro-intestinal tract by monilia. The patient's condition was apathetic, and he gave no evidences of pain or other symptoms referable to the musculatory system.

The postmortem disclosed the changes seen in severe anemia due to malaria, besides other lesions without special interest. The musculature presented no gross evidence of sarcocysts for these were too

Fig. 1.—Section of muscle, Case 2 "Ali," showing oblique section of muscle and a sarcocyst which through the obliquity of the section appears as two cysts.

small to be recognized by the naked eye. On inspecting the sections of tissues from the mouth, lips and tongue, sarcosporidia were encountered in one out of four blocks from the latter.

DESCRIPTION OF THE SARCOSPORIDIA

The section presented an elongated stippled body faintly encapsulated. The sarcocyst was not definitely imbedded in a muscle fiber, although lying parallel to the fibers near by and having a diameter or width two to three times that of the muscle fiber. The length was

two, three or four times its width, but there was some obliquity to the section thus shortening its real length.

The stippling was due to the nuclei of the sporoblasts which were slightly larger than those encountered in my first case, but much smaller than those of the sarcocyst of the sheep, hog, horse or rat, and on the whole were of the small type which I have described from the oppossum, hawk, guinea-pig and man (Darling, 1915). There was no evidence whatever of degeneration or inflammatory change, not even the slightest in the neighborhood of the sarcocysts.

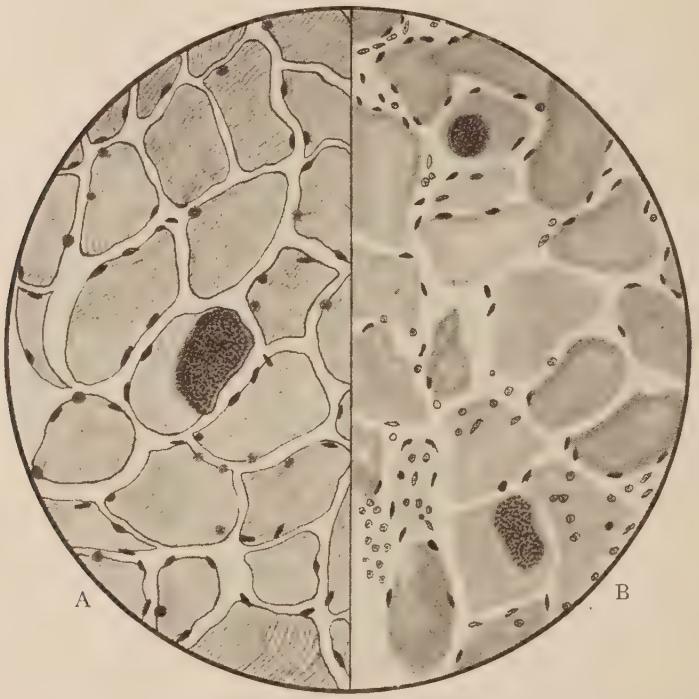


Fig. 2.—Section of muscle, Case 1, J. H.; part (*A*) represents a piece of muscle taken July 2; part (*B*) represents a fragment removed July 13. The necrosis of the fibers and the cellular changes are due to typhoid fever and probably not to the presence of the sarcocyst.

DISCUSSION

Nothing is known of the man's habits previous to his admittance to the hospital. He had been an estate coolie and a bullock cart driver. He was also a mohammedan. From this we can state that like most East Indians he was practically a vegetarian, and that his diet consisted almost wholly of boiled rice, milk, some fruit and occasionally though rarely a bit of goat's flesh, chicken or fish. Meat could be almost entirely put out of his dietary. In our ward he got chicken, rice and

milk. There is no likelihood of his ever having tasted or eaten raw meat or fish. The source of the infection then is unknown.

Scott has published some interesting work on the epidemiology of sarcocyst infection in sheep and it is to be hoped that the natural mode of infection of this parasite will before long be cleared up.

Scott's recent paper (1918) on the seasonal incidence of sarcocyst infection in Wyoming is suggestive of the possibilities for the truth of my view that sarcosporidiosis as well as leishmaniasis and certain other infections are examples of parasitological blind alleys, and that sarcosporidiosis is very likely an infection by some sporozoon, very likely Neosporidia derived from insect or invertebrate through the contamination of food or drink, directly or indirectly, through droppings, and that the sporozoon after gaining the musculature of its strange host is unable to continue further its life cycle and escape from a compromising position.

Papers Cited

Baraban and St. Remy, 1894.—Sur un cas de tubes psorospermiques observés chez l'homme. Compt rend. soc. biol., Paris, (X), 1: 201-202.

Darling, S. T., 1909.—Sarcosporidiosis with Report of a Case in Man. Arch. Int. Med., 3: 183-192.

1915.—Sarcosporidia Encountered in Panama. Jour. Parasitol., 1: 113-120.

Scott, J. W. 1918.—Notes and Experiments on *Sarcocystis tenella* Railliet. Jour. Parasitol., 5: 45-60.

CONCENTRIC BODIES, PROBABLY OF PARASITIC ORIGIN, IN THE AUSTRALIAN SEA MULLET, *MUGIL DOBULA*

J. Burton Cleland
Department of Public Health, New South Wales

The specimen, the subject of this note, was submitted to us by the Fisheries Department of the State of New South Wales in May, 1916.

The lesions consist of numerous small scattered areas, distributed through the musculature, composed of concentrically arranged cells, the larger areas showing degenerated centers. These pathological areas in appearance resemble somewhat the well-known cell-nests of a squamous epithelioma of man.

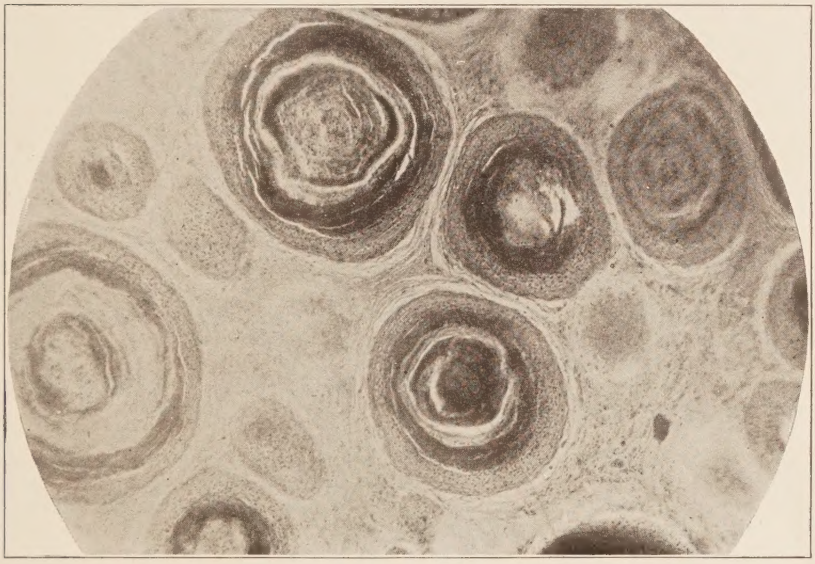
No parasitic bodies, protozoal or helminthic, could be recognized in the lesions, unless the cells composing the areas are themselves parasitic, which does not seem likely to be the case. Nevertheless, it is reasonable to assume that the lesions are due to the reaction to a parasite of some kind, past or present.

The notes are submitted with the object of calling attention to the condition and of elucidating, if possible, a satisfactory explanation of the appearances met with. The writer will be glad of any information in reference to the condition, which may perhaps be well known.

Description of the specimen.—Throughout the musculature are small, scattered, reddish, granular areas, the largest about the size of rice grains, many being smaller. These appear on the inner side of the ribs as small warty areas, and also extend on the back plate of the gills (all that remain of these, the fish having been cleaned for market). In places where the scales are thin, minute reddish spots, evidently due to the same condition, can be seen through them.

Microscopically, the affected tissue is found to be occupied by concentrically arranged masses of cells, the masses being usually spherical, but sometimes somewhat irregular. The smallest is about 60μ in diameter, whilst the largest reaches just over 1 mm. in diameter, the most frequent size being about 0.6 mm. The larger masses consist externally of layers of concentrically arranged elongated cells with medium-sized vesicular nuclei. The average diameter of these cells is about 8μ. These outer layers are succeeded internally by further layers of concentric cells, in which, probably from degeneration, the nuclei are condensed into small dark bodies about 2μ in size. In old lesions the center of the body stains deeply with iron hematoxylin. It is evidently much degenerated, the individual cells being mostly

indistinguishable, and is apparently somewhat calcified in part. Occasionally, in the center of the body are indefinite masses of black pigment. The smaller bodies represent presumably earlier lesions; some show the "outer" type of cell only. No central parasite could be recognized in either the early or late lesions. The lesions are found amongst the muscle tissues in the deeper parts, and even in the subcutaneous tissues they are seen in proximity to muscular masses.



The accompanying photograph illustrates the appearances presented better than a description in words.

NOTES

The Molteno Institute for Research in Parasitology has been founded at the University of Cambridge (England) by a gift of $150,000 for building and equipping the laboratory and for providing an income to maintain it. The generous gift comes from Mr. and Mrs. P. A. Molteno and is in response to an appeal showing the need of such an establishment which was prepared by Dr. G. H. F. Nuttall, well known for his work in parasitology and for his editorship of the English publication bearing that name. This appears to be the first institute established specifically for this purpose.

The genus Demodex, monographed by Stanley Hirst, is the title of the first number of Studies on Acari, published by the British Museum (London). This work presents a wealth of new and important data on the structure, biology and classification of a group hitherto little known.

An investigation of the Louse Problem by William Moore and A. D. Hirschfelder which appeared recently in Research Publications of the University of Minnesota, deals with methods of rearing, controlling and destroying lice, and pathological effects of the bite of the clothes louse. It is an exhaustive and critical study that deserves to be widely known.

PLATE VIII

EXPLANATION OF PLATE VIII

Fig. A. The mature sporocyst of *Leucochloridium problematicum*.

Fig. B. The mature sporocyst of *Leucochloridium macrostomum*, after Carus.